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THE CONTROLLED RAPID INDUCTION OF PORTAL CIRRHOSIS
AND PORTAL HYPERTENSION IN DOGS

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES
IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE
OF MASTER OF SCIENCE

FACULTY OF MEDICINE
DEPARTMENT OF SURGERY

by

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A B S T R A C T

There are numerous methods of producing portal cirrhosis in dogs, but all require periods ranging from one to several years. The objective of this project is to demonstrate a rapid method of producing portal cirrhosis and portal hypertension in dogs using carbon tetrachloride as the etiologic agent and serum Glutamic-Oxalacetic Transaminase (SGOT) estimations to monitor the process. The carbon tetrachloride was given orally in gelatin capsules each containing one milliliter of the drug. Initially very small quantities were given; for example, 1.5 ml. every second to third day, but the dosage was gradually increased. The dosage was determined by SGOT estimations which were interpreted as evidence of the extent of hepatic necrosis. The aim was to limit necrosis so that the animal's health would deteriorate only gradually as a result of increasing hepatic necrosis. The dogs received an average of 228.5 ml. of carbon tetrachloride over periods ranging from 87 to 139 days. The technique of administering the drug was to personally insert the capsules into the hypopharynx of each dog thereby ensuring ingestion in every instance. The following tests were performed at intervals during the induction of cirrhosis as a guide to the state of liver function and general health: Serum Alkaline phosphatase;

bromsulphalein retention (BSP); serum bilirubin; serum cholesterol; plasma proteins; cephalin-cholesterol flocculation; hemoglobin; hematocrit; blood urea nitrogen (BUN). The general appearance, weight, and abdominal palpation were also noted. The final determination of hepatic status was made by performing laparotomies on the experimental animals to obtain liver biopsies for histopathological examination. The peritoneal cavity and its contents were examined for any abnormality that may be associated with portal cirrhosis and portal hypertension. Portal pressure was measured in veins of the ileal mesentery. During the course of the experiment the dogs were kept on a standard diet reinforced with glucose.

At laparotomy all the dogs showed varying stages of portal cirrhosis from mild to severe. All the dogs had portal hypertension and three exhibited ascites. The development of a collateral circulation was not seen. Liver function tests showed progressive dysfunction. Controls for all the parameters used were calculated from findings on 26 normal dogs. The great majority of the liver function tests were significantly altered. Microscopic examination of the livers at laparotomy demonstrated a tawny discoloration, nodularity and an increased consistency. Histopathology interpreted by means of hematoxylin and eosin, and reticulum staining

of biopsies demonstrated portal cirrhosis in most of the dogs.

It is concluded therefore that portal cirrhosis may be induced in experimental dogs using carbon tetrachloride orally over a comparatively short period of three to five months with exceedingly low mortality. The factors necessary are:

1. The use of SGOT as a monitor to determine the amount of hepatic necrosis produced by the intermittent ingestion of carbon tetrachloride. The dose of carbon tetrachloride was determined by SGOT levels. It is felt that this controlled production of hepatic necrosis accounts for the low mortality rate.

2. The method of giving the carbon tetrachloride is important for it is necessary to know the exact quantity of the drug that is ingested by the dog in every instance. The technique of using recently prepared carbon tetrachloride in one ml. gelatin capsules, introducing the capsules into the pharynx of the animal to ensure ingestion in every case fulfills the above mentioned requirements admirably.

3. The diet should be augmented with a milk-syrup mixture to ensure an adequate carbohydrate content during the induction of cirrhosis.

It is felt that with more experience in this technique, clinical cirrhosis may be produced in dogs in the even shorter period of two to three months. This

would make the experimental study of the etiology, biopathology and therapy of portal cirrhosis a more practical, less time consuming and less expensive problem.

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INTRODUCTION

Cirrhosis of the liver is a common disease (United States Public Health Service, report on vital statistics, 1957) becoming increasingly more common and unfortunately presents clinically at a late stage in which the process is irreversible and the patients' days numbered by the disease itself or its dangerous complications.⁽¹⁾ It is suggested that an increased awareness by the medical profession concerning early cirrhosis of the liver as a cause of ill health will assist greatly in increasing the understanding of the pathogenesis of this disease, which has become the fourth most frequent cause of death in persons above the age of 40 years in the United States of America.⁽²⁾

Kleckner in his recent book on cirrhosis of the liver regards needle biopsy generally as the best single available diagnostic method in diseases of the liver including cirrhosis, stressing the fact that its diagnostic and practical value far exceed its limitations and potential risk, but adds that it should always be regarded as a valuable supplementary diagnostic tool rather than a substitute for clinical judgement.⁽³⁾

This thesis is concerned with a method of producing experimental portal cirrhosis and portal hypertension rapidly in dogs, with the aim of providing investigators with a reliable and successful technique with which to study cirrhosis of the liver

in its many and varied aspects.

Cirrhosis received its appellation at the hand of Rene Theophile Hyacinthe Laennec in 1826. The word is derived from the Greek "KIRRHOS" meaning tawny or orange colored, and although it has maintained its popularity universally, it is recognized that the word simply represents a common name of a group of different pathological states of the liver, with distinctive clinical and pathological features.

What is cirrhosis? Many definitions of this condition exist suggesting a lack of knowledge concerning both etiology and pathogenesis. It has been a controversial problem for many years and the subject of a series of group studies aimed at its classification and clarification.^(4,5)

Mallory,⁽⁶⁾ in 1911, defined cirrhosis as a chronic progressive destructive lesion of the liver associated with reparative activity and contraction on the part of the connective tissue. He classified cirrhosis into five different types: (1) Toxic, (2) Infectious, (3) Pigment, (4) Syphilitic, (5) Alcoholic. Later on other writers felt that the morphological constituents of cirrhosis were nodular regeneration, fibrosis, and hepatocellular necrosis.⁽⁵⁾

Other common pathological features present in cirrhosis and varying in extent and intensity were described as:

1. Particular degenerations; for example, hyalinization, steatosis, necrosis, pigment deposits.
2. Cellular infiltration.
3. Thickening and proliferation of reticulum.
4. Formation of pseudobiliary ducts or proliferation of interlobular ducts.
5. Sclerosis, necrosis, and regeneration of blood vessels.
6. Enlargement or atrophy of the liver.

The International Society for Geographic Pathology, in 1931, accepted the definition of cirrhosis as an alteration of the liver in which (grossly) the presence of nodules is associated with an increase of the connective tissue. It also accepted that microscopic examination further discloses hepatocellular degeneration, necrosis and regeneration associated with chronic inflammation and fibrosis. To this definition is frequently added the distorted reconstruction of the lobular architecture. (7,8,9)

Moon,⁽⁷⁾ in 1932, considered cirrhosis synonymous with chronic diffuse hepatitis and this attitude was generally accepted. The cirrhotic process consisted of degeneration and destruction of hepatic cells followed by regeneration and by fibrous tissue

proliferation. If destruction was limited in extent, no marked structural or functional disturbance resulted. Varying degrees of minor injury lead to varying degrees of chronic hepatitis not easily classified. Progressive repeated destruction and regeneration resulted in nodules with abnormal circulatory relations. The normal lobular pattern was obliterated and the hepatic architecture altered. Destruction of architectural pattern was noted as a feature and differential criterion of portal cirrhosis. Moon stated that no single causative factor was responsible for cirrhosis and that the etiology would be found to be as variable as are the agents which, singly or in combinations, may cause chronic diffuse progressive inflammation of the liver. This statement seems as true now as it was when enunciated nearly 30 years ago.

In a classic publication in 1943, Karsner⁽⁸⁾ incorporating some of the conceptions of predecessors, considered fibrosis as the pertinent pathological feature of cirrhosis. His classification of cirrhosis included, (1) Laennec's cirrhosis, (2) Fatty cirrhosis, (3) Pigmentary cirrhosis, (4) Biliary cirrhosis, (5) Postnecrotic cirrhosis, (6) Congestive cirrhosis, (7) Syphilitic cirrhosis, (8) Zooparasitic cirrhosis, (9) Tuberculous cirrhosis, and (10) Cirrhosis of the

Lipoidoses. He did not consider cirrhosis to be a representation of a chronic infectious process. He suggested that an ideal classification of cirrhosis would be etiological. This is not necessarily so for the numerous etiological factors may result in similar cirrhotic patterns while differences in the resulting transfiguration of hepatic architecture may depend more on degree and duration of the etiological insult as well as the subject's state of health and resistance at the time. This is strongly reminiscent of the old analogy of the "seed" and the "soil" in determining the prognosis of an infection.

In 1951, the Registry of Hepatic Pathology of the Armed Forces Institute of Pathology classified cirrhosis as (1) Portal, (2) Postnecrotic, (3) Biliary, or (4) Type Undetermined. The Registry also recorded histopathological criteria for cirrhosis as follows:

1. Portal Cirrhosis. There is an increase in the number of small bile ducts and the amount of portal collagenous tissue with definite pseudolobular formation. It is regarded as a diffuse hepatic disease involving all of the portal canals in a uniform degree.

2. Postnecrotic Cirrhosis. In this condition there are broad areas of scarring, increased bile ducts, and pseudolobular formation. Some portal canals are

normal or only slightly altered.

3. Biliary Cirrhosis. There is an increase in the amount of portal collagenous tissue and in the number of small bile ducts, sometimes with pseudolobular formation but usually with a centrally located efferent vein and small bile "thrombi" in the centrolobular sinusoids. In the late stages it may be indistinguishable from portal cirrhosis.

4. Type Undetermined. Those cases of cirrhosis showing pseudolobule formation but which do not fulfill the criteria for any of the three above mentioned types.

A comprehensive and contemporary classification of cirrhosis was proposed by Watson in 1952. (10) He recognized two main types, one in which a fatty liver is the main pathogenetic feature, and another in which cirrhosis is non-fatty during its pathogenesis.

A still newer concept of cirrhosis was evolved at the Fifth Pan-American Congress of Gastroenterology held in Cuba during January of 1956. (4) It was defined anatomically with an additional clinical concept. Its anatomic definition stipulated that all parts of the liver are involved without necessarily affecting each lobule, cellular necrosis at some stage of the disease, nodular parenchymal regeneration, diffuse fibrosis and disorganization of the lobular architecture with connective tissue bands uniting central lobular zones with the portal tracts. The clinical concept

includes, (1) chronic disease, and (2) liver cell failure and portal hypertension of variable severity.

This Congress further classified cirrhosis on a morphologic, etiologic and functional basis. The three morphologic types were, portal, postnecrotic and biliary (with or without extrahepatic biliary tract obstruction).

The etiological factors accepted were:

- (1) Malnutrition.
- (2) Ethyl alcohol.
- (3) Viral hepatitis.
- (4) Obstruction of the extrahepatic biliary tract.
- (5) Cardiac failure.
- (6) Hemochromatosis.
- (7) Congenital syphilis.

Also considered were:

- (8) Toxic agents; for example, carbon tetrachloride, trinitrotoluene.
- (9) Granulomatous lesions; for example, brucellosis, tuberculosis, and sarcoidosis.
- (10) Helminthic infestations; for example, schistosomiasis.
- (11) Disturbances in copper metabolism.

The functional aspects of the classification included:

- (1) Liver cell failure, as demonstrated by clinical

states, such as, jaundice, ascites, and coma, as well as by abnormal liver function tests.

(2) Portal hypertension as shown by splenomegaly, esophageal varices, and a demonstration of a raised portal pressure.

(3) Activity of the disease, whether progressing, regressing, or stationary.

Although the above classification is excellent in its grip of the scope of the problem of cirrhosis, certain obstacles still exist which tend to make its use generally a trifle premature. These include the nonspecificity of hepatic function tests and the important fact that in most conditions the etiology is purely speculative. It is, however, an exceedingly valuable classification and is to be recommended, for in this manner a great body of organized data concerning cirrhosis will become available from which much useful information will derive. Also in 1956, Schaffner, Popper, and Dalla Torre proposed their functional therapeutic classification of cirrhosis based on morphological criteria of the progression of the disease, the architecture of regenerative nodules, portohepatic vascular anastomoses, and the amount of hepatocellular damage.⁽¹¹⁾ They correlated hepatocellular degeneration with jaundice, gastrointestinal

bleeding, and abnormal hepatic function tests and noted that the only clinical feature that correlated with advanced cirrhosis was splenomegaly. Progression of cirrhosis was noted frequently when the patient displayed jaundice, ascites, splenomegaly, spider angioma, palmar erythema, and raised values of serum gamma globulin and thymol turbidity. But these unfortunately are the stigmata of advanced cirrhosis when very little can be done to halt or reverse the fatal process. While it may be necessary to arrange separate clinical, pathological, and histological classifications of cirrhosis in the absence of an established etiology and reliable liver function tests, Kleckner makes an attempt to separate the clinical, gross morphological, and histopathological nomenclature of cirrhosis.⁽³⁾ Clinically, he divides cirrhosis into the following sub-divisions:

1. Portal.

This is commonly due to malnutrition and viral hepatitis.^(12,13,14) The early stage is characterized by nondescript symptoms, such as flatulence, morning nausea, vomiting, fatigue, weakness, anorexia, and sexual impotence. Physical findings include ascites, abdominal distention, malnutrition, loss of weight, feminizing features, jaundice, gastrointestinal haemorrhage, spider angioma, palmar erythema, hepatosplenomegaly, pedal

edema, collateral venous circulation, and esophageal varices. Jaundice associated with cirrhosis represents either transient or terminal hepatic insufficiency. The common laboratory findings are retention of bromsulphalein, hypoalbuminemia, hyperglobulinemia, positive hepatic flocculation tests, leukocytosis and abnormal plasma prothrombin values.

2. Postnecrotic.

This usually follows viral hepatitis or hepatotoxic agents. (15,16,14) This represents vigorous parenchymal regeneration following massive hepatic necrosis. The clinical picture is not characteristic. It is diagnosed only by sufficient hepatic biopsy specimen. The symptoms are usually indicative of hepatic insufficiency. Hypersplenism and bleeding exophageal varices are common. It is more common in females, often follows menarche, pregnancy, and menopause. The clinical course tends to be rapidly progressive. Laboratory findings include marked hypergammaglobulinemia, leukopenia, positive hepatic flocculation values, low plasma cholesterol ester and cholinesterase values, thrombocytopenia, increased serum bilirubin, transaminase, and iron and hypoprothrombinemia.

3. Biliary

(a) Primary, synonymous with cholangiolitic cirrhosis may be indistinguishable from congenital atresia

of the intrahepatic bile ducts. The etiology is unknown. This group is usually confined to middle-aged females. Common complaints include pruritis, abdominal pain, weakness, intolerance to fatty foods, loss of weight, osseous pain, steatorrhea, and menstrual irregularities. Physical findings include cutaneous melanosis, excoriations, jaundice, xanthomata, hepatosplenomegaly, lymphadenopathy, clubbed fingers, low-grade fever, and alopecia. Laboratory findings are consistent with obstructive jaundice. The diagnosis is essentially clinical. Obstructive lesions of the extrahepatic biliary system must be excluded.

(b) Secondary, due to extrahepatic biliary obstruction.⁽¹⁷⁾ Clinical and laboratory features are similar to those observed in cases of primary biliary cirrhosis.

4. Hemochromatosis

The etiology of this obscure condition is unknown. It usually affects males in the fifth or sixth decade of life. Cirrhosis, diabetes mellitus, cutaneous melanosis, and hypogonadism are common clinical features.

5. Hepatolenticular Degeneration

This condition occurs in consanguineous families. There are hepatic, lenticular and hepatolenticular clinical forms. The clinical picture includes dementia, extrapyramidal neurologic involvement and malnutrition. Cirrhosis may occur late. The condition is thought to be associated with an abnormal storage of

copper. The disease is relentlessly progressive. (18)

6. Cirrhosis in Infants and Children

This includes cirrhosis associated with galactosemia, congenital fibrocystic disease of the pancreas, kwashiorkor, glycogen storage disease, sickle cell anemia, veno-occlusive disease and erythroblastosis fetalis.

A gross morphological classification of cirrhosis includes:

1. Portal, in which the regenerative nodules are uniform in size having a diameter of 0.5 centimeter or less. The gross appearance is granular with the color varying from yellow to gray.
2. Postnecrotic, in which the regenerative nodules are large or lobular, The color may be yellow, green, or brown.
3. Biliary, which resembles portal cirrhosis but in which the color is usually green.
4. Hemochromatosis, which resembles portal cirrhosis but in which the color is usually reddish-brown.

Finally, Kleckner classifies cirrhosis histopathologically into four main groups, Portal, Postnecrotic, Biliary, and Hemochromatosis, stating that to adequately fulfill a histological diagnosis of cirrhosis, nodular parenchymal regeneration and increased amounts of fibrous connective tissue in the form of stroma must be present.

The following is a brief description of the four histopathological varieties:

1. Portal

There is uniform alteration of the hepatic architecture.^(7,19) The hepatic veins are located in various areas of the nodule. Regenerative nodules are uniform, small, and separated from each other by zones of fibrous connective tissue. The regenerative nodules may be the site of fatty infiltration, necrosis, polymorphonuclear leukocytosis, and bizarre hepatic cells. An increased number of bile ducts are present in the internodular stroma, infiltrations of leukocytes and compression of small venules and blood vessels.

2. Postnecrotic

This is characterized by irregularly shaped regenerative nodules usually larger than one centimeter in diameter and separated by broad bands of fibrous connective tissue. Bizarre hepatic cells, focal necrosis, monocyctic infiltration, rarity of fatty infiltration are some of the histological findings present. The internodular stroma contains an increased number of bile ducts, infiltration of monocytes, compressed hepatic veins, and inflammatory changes in the blood vessels.

3. Biliary

Histological diagnosis depends on the presence of the usual criteria of portal or postnecrotic cirrhosis

in addition to cholestasis. There is no characteristic distinction between primary and secondary biliary cirrhosis from a histological point of view. There is also a marked proliferation of fibrous connective tissue in the portal spaces together with abundant infiltrations of lymphocytes.

4. Hemochromatosis

All the features of portal cirrhosis are present in addition to large amounts of hemosiderin in the hepatic cells, stroma, bile duct epithelium, and Kupffer cells.

Popper and Zak (20) in an excellent paper in 1958, surveyed the pathophysiologic phenomena which characterized cirrhosis and then utilized them to arrive at a workable definition based on clinicopathological correlation and a suitable basis for classification.

The basic problem, still unsolved, is whether cirrhosis is a primary disturbance of the hepatic cells with reactive connective tissue formation (7,8,9,21,22) or a primary mesenchymal lesion, inflammatory in character, in which the epithelial alterations are secondary to the mesenchymal changes, particularly to the scarring. (23,24)

The histopathological features of cirrhosis are numerous and due in most instances, it appears, to hepatocellular degeneration and necrosis. Some of the changes are due to the cause of the cirrhosis while

others are induced by the cirrhotic process itself; for example, fibrosis causing attenuation of the blood supply and further hepatocellular necrosis. In this manner a vicious cycle is established in which the pathological process continues although the original cause may no longer be present or active. The following include most of the histopathological features present in cirrhosis:

1. Homogeneous cytoplasmic coagulation and single cell necrosis. This is associated with an accumulation of mononuclear cells and circumscribed collapse of the framework.

2. Focal granular clumping of the cytoplasm. The clumps aggregate to form a ramified perinuclear acidophilic body which has been designated by Mallory⁽²⁴⁾ as "alcoholic hyaline". This form of degeneration is associated with focal aggregation of segmented leukocytes.

3. Centrolobular necrosis occurs in chemical poisoning, other types of toxic hepatic injuries, and passive congestion.

4. Ischemic degeneration and necrosis developing in the centre of the nodule due to constriction of the blood supply resulting in oxygen deficiency which interferes with enzymatic processes.⁽²⁵⁾

5. The topical effects of bile on hepatic cells resulting in a reticular arrangement and bile pigmentation of the hepatic cytoplasm.

6. Massive necrosis may be due to the original cause of the cirrhosis or the cirrhotic process itself. The collapsed areas produce the stress or break fissures in the surrounding parenchyma.

7. Fatty metamorphosis may precede or complicate cirrhosis. The site of accumulation of the fat depends frequently on the cause of the cirrhosis. (26,27) Differences in the vascular supply between human and animal livers may account for the manner in which species vary in the hepatic reaction to cirrhosis-evoking agents. The work of Elias and Popper has done much to elucidate this. (28)

8. Regeneration. This is a process which normally takes place in the mammalian liver, but which becomes accentuated in the vicinity of focal or sub-massive necrosis. The interruption of the liver cell plates seems to provide a growth stimulus. Regeneration is reflected morphologically both cytologically (increased cytoplasmic basophilia, nuclear and nucleolar enlargement and multiplication - polyploidy) and by hepatic architectural alteration (formation of liver cell plates more than one cell thick). Multinuclear giant cells are not infrequent. Accentuated regeneration in a circumscribed area initiates the formation of nodules, which distort the hepatic architecture exerting pressure on the surrounding parenchyma and stroma which in turn becomes compressed into connective tissue

septa. In the nodules the liver cells lose their original radial alignment and instead converge towards the centre of the nodule where sinusoids become transformed into an efferent vein. As the nodule grows, the portal vein branches and bile ducts grow into it to produce a secondary "lobulization" which may eventually lead to secondary formation of normal central veins and portal tracts which are smaller. However, numerous regenerative nodules may be examined without demonstrating any evidence of this rearranged vascularity and the pathologist may have difficulty in recognizing the vascular arrangement of the nodules. The difference in color and appearance of isolated nodules may be the result of local necrosis from disturbances of blood flow or focal bile stasis, but usually reflects metabolic autonomy which makes such nodules susceptible to malignant change. The high incidence of neoplasm associated with cirrhosis was noted earlier in this chapter.

9. Ductular cell reaction. Excessive proliferation of the ductular cells is noted in cirrhosis. This may be recognized by the presence of increased proliferation of bile ductules or the presence of ductular cells singly or in clumps. The origin of the ductular cells whether from hepatic cells or biliary epithelium is

controversial, but whatever its origin the ductular cell reaction is a response of the liver to injury of various kinds and a common finding in cirrhosis.

10. Fibrosis. This is due to an increase in the hepatic collagen and reticulum and may be the result either of (1) approximation (collapse) of preformed stroma after disappearance of the parenchymal cells, or (2) new formation, or (3) both. Excess fibrous tissue, a constant finding in cirrhosis, may present itself in the following configurations.

- (1) Focal intraparenchymal fibrosis, usually following focal necrosis.
- (2) Central lobular fibrosis, occurs mainly after passive congestion following intoxication by some drugs; for example, carbon tetrachloride.
- (3) Portal fibrosis following portal inflammation, such as pericholangitis, extrahepatic biliary obstruction.
- (4) Periportal fibrosis with diffuse widening of the portal tract and stellate enlargement produced by extensions of short septa in various directions from the portal tract.

(6) Septal fibrosis seen as septa traversing the lobular parenchyma. They either surround lobules as a perilobular fibrosis or extend into them as a stellate fibrosis. These septa may connect with each other dividing lobules irregularly into nodules.

(7) Postcollapse fibrosis results from sub-massive and massive collapse and is characterized by broad connective tissue bands which are apparent to the naked eye.

Either some or all of the above findings are present in cirrhosis depending on the degree and type of etiological factor, as well as the pathway which the cirrhotic process has followed. This latter factor is important, for long after the cause of the cirrhosis has ceased to exist, the mechanical effects of the disease may perpetuate the condition and imitate irreversibility of the process.

Of great interest is the fact that different types of cirrhosis may be produced by the same etiological factor. This is evident where examination of one section of the organ demonstrates, say postnecrotic cirrhosis while another area displays a portal or septal variety of cirrhosis. In the project on which this thesis is

based, carbon tetrachloride, a chemical which usually produces postnecrotic cirrhosis, has been used to produce a diffuse portal cirrhosis successfully. It seems justifiable to postulate, therefore, that cirrhosis is simply a histopathological reaction to hepatocellular injury, the final result being dependent on a multiplicity of factors, including types, degree, and duration of etiological factor, diet, previous hepatic disease, concomitant diseases: for example, alcoholism, and the manner in which the hepatic vasculature is affected. This last factor is exceedingly important, both in determining the type of cirrhosis produced, and its functional effects; for example, hepatic insufficiency, portal hypertension. There is no way of predicting the manner in which the vascular system of the liver will be affected. Numerous investigations have demonstrated beautifully the precise vasculature of the liver and the ways in which it can be compromised. (30,31,32,33,34,21,22) These have done much to improve the understanding of the functional pathology and complications of cirrhosis. Much credit is due to the outstanding work of Popper and Elias in elucidating the microanatomy of the liver and the histogenesis of cirrhosis, using a three dimensional analysis and a statistico-geometric method. (28,35,36,34,37)

It appears that the branches of the portal vein are the first vessels to be affected by the cirrhotic process. The portal veins and hepatic arteries are well protected in their connective tissue tracts but not so the hepatic vein tributaries, which are consequently compressed and distorted by the fibrosis and nodular regeneration. This postsinusoidal compression interferes with the drainage of blood from the liver and is one of the main causes of portal hypertension in cirrhosis.(33,34)

Since small nodules compress veins more effectively than larger ones, they are more frequently associated with portal hypertension. Injection techniques have demonstrated parasinusoidal communications between branches of the portal and hepatic veins which shunt blood in a portohepatic direction by-passing the parenchyma. Together with the extrahepatic porto-systemic anastomoses (38), they divert blood from the hepatic parenchyma. Presinusoidal anastomoses between portal vein and hepatic arterial branches are also present in cirrhotic livers. This contributes further to portal hypertension and also to diversion of portal blood from the parenchyma which adds further to the general organic insult.

Neither the factors which bring about the transformation of the normal liver into a cirrhotic organ nor the dynamics involved are properly known, but it seems likely that an important part is played by the disorganization of the lobular architecture and the deleterious changes wrought in the vascularity of the structure.

The aim of this project was to develop a rapid technique of producing portal cirrhosis and portal hypertension in dogs and to supply further information concerning its histopathogenesis and biochemistry.

CHAPTER I. PART A

TECHNIQUE OF PRODUCING CIRRHOSIS

A random selection of ten mongrel dogs weighing between 12 and 24 kilograms was made. All were submitted to a battery of blood and liver function tests and found to be within normal limits. A control for all the parameters was calculated from the findings on 26 healthy dogs (See Table 2).

A normal SGOT level was established so that this test could be used to indicate the degree of hepatocellular necrosis that took place while the cirrhotic process developed and thereby act as a guide to the quantity of carbon tetrachloride administered. The carbon tetrachloride was administered orally in gelatin capsules which were prepared in the Pharmacy of the University of Alberta Hospital. Initially, exceedingly small doses of carbon tetrachloride were given; e.g., 1.5 milliliters twice weekly. Exceedingly small doses of carbon tetrachloride were used initially because the studies of Gardner and Groves (39) on the pathological histology of experimental carbon tetrachloride poisoning demonstrated clearly that although the smallest dose of carbon tetrachloride which would produce liver necrosis had not been definitely determined, 0.176 milliliters per kilogram body weight was found sufficient to produce a central hepatic necrosis in dogs.

The dose was gradually increased, the quantity being determined by the SGOT level, until some 10 milliliters were being given on alternate days. When the SGOT level ascended, this was interpreted as increasing hepatocellular necrosis and the dose of carbon tetrachloride accordingly regulated. In this way, it is argued, the rate of necrosis was controlled and never allowed to proceed too rapidly, thus preventing any great mortality from acute massive liver necrosis. The aim was to limit the necrosis so that the animal's health would deteriorate only gradually. The drug was given over a period ranging from 87 to 139 days, each dog receiving an average of 228.5 milliliters of carbon tetrachloride. The following table gives a detailed account of the quantity of carbon tetrachloride supplied over a given period to each of the 10 dogs.

<u>Dog:</u>	<u>Amount of CCl₄:</u>	<u>Time:</u>
K25	242.50 ml.	138 days
K26	237.00 ml.	138 days
K27	251.25 ml.	139 days
K28	242.00 ml.	138 days
K29	159.25 ml.	110 days
K30	270.00 ml.	99 days
K31	280.75 ml.	112 days
K32	140.25 ml.	82 days
K33	243.25 ml.	102 days
K34	218.75 ml.	95 days

The technique of administering the drug was to insert the capsules containing the carbon tetrachloride into the hypopharynx of each animal thereby ensuring ingestion of the drug in every instance. Furthermore the animals were observed for a period following the administration of the drug so that any regurgitation of capsules would be noticed and replaced. This rarely occurred. The dogs soon became accustomed to the method and accepted it often with great excitement and enthusiasm.

Carbon tetrachloride was chosen as the etiological factor because it has been used successfully by numerous investigators in the past to produce cirrhosis in a variety of experimental laboratory animals. (40,7,41,42,43,44,45,46,47)

Numerous other methods of producing experimental cirrhosis are available, however, and these include the use of diets deficient or overloaded by various components. (48,49,50) Inorganic poisons; e.g., phosphorus, arsenic, lead, manganese, and copper (7) have also been used successfully in producing experimental cirrhosis.

The place of alcohol in the etiology of cirrhosis has still not been determined but is probably a contributing or predisposing factor. (51,52,53)

Bacterial infection ⁽⁵⁴⁾ and the following miscellaneous organic substances have also been used to produce cirrhosis - Butyric, Valerianic, and Acetic acids, Cholesterol, Tobacco, Amonium carbamate, Chloroform, Formaldehyde, Sulphuric acid, Ricin, Bile, Commercial peptone, Aniline, Toluidine, Hydrazine, and Cincophen.

Moon⁽⁷⁾ quotes only a few agents as having satisfied the rigid criteria for Laennec's Portal Cirrhosis, and these include Phosphorus and Alcohol, Manganese chloride and Phenylhydrazine, Carbon tetrachloride, Tars, Bacterial infections, and combinations of infections with other agents.⁽⁵⁴⁾ His criteria for Laennec's cirrhosis were:

1. Distortion of the hepatic architecture.
2. Alteration of the lobular pattern.
3. Islands of regenerating cells forming nodules.
4. Disarrangement of the circulation of the liver.

Because of the importance of diet in cirrhosis, it was decided to give the dogs a full diet supplemented daily by a syrup and milk mixture as well as a moderate quantity of meat.

Laparotomies were performed on the 10 dogs three to five months after starting the experiment, irrespective of the condition of the animals so that the liver could be observed, a biopsy taken and the

peritoneal cavity further examined for evidence of portal hypertension or any other abnormality. During the period of induction of cirrhosis, the dogs were observed clinically, weighed regularly, and various biochemical blood tests were performed intermittently.

CHAPTER I, PART B.

SGOT MONITORING OF NECROSIS

It has been demonstrated adequately that SGOT activity is a highly specific index of hepatocellular injury. (55,56,57,58,59,60) The height and duration of increased SGOT activity was found to be proportional to the amount of carbon tetrachloride administered as well as to the severity of liver cell damage, and there appeared to be a correlation between the extent of centrilobular zonal necrosis and the height of SGOT levels attained. Serum alkaline phosphatase for example, does not have the same degree of sensitivity that SGOT activity possesses in reflecting hepatocellular injury. It was thus demonstrated that SGOT may be a useful guide of the extent of hepatocellular necrosis occurring and also in following the progress of restoration of hepatocellular integrity following acute injury. It appeared therefore that this would be an excellent method of monitoring the amount of hepatocellular necrosis produced by carbon tetrachloride while attempting to induce cirrhosis in dogs. In this way the necrosis could be controlled and mortality from massive necrosis limited. It is in great part due to this technique that at the time of laparotomy the dogs apart from the two which succumbed earlier were in moderately good clinical health.

Goldstein, Seligson and Nemir⁽⁶¹⁾ demonstrated the specificity of SGOT as an index of hepatocellular necrosis. They showed markedly raised SGOT levels in hepatocellular hepatitis, moderately raised levels in cholangiolytic hepatitis, and only slightly raised or normal levels in extrahepatic obstructive jaundice.

The cause of raised SGOT levels in obstruction is probably due to mild liver cell damage and regurgitation of excreted transaminase into the circulation.

Wroblewski suggests the following mechanisms for alteration in serum or plasma enzymes including SGOT and serum alkaline phosphatase.⁽⁶²⁾

1. Necrosis of tissue results in release of intracellular enzymes which find access to the blood stream.
2. Necrosis and inflammation of tissue results in release of intracellular enzymes which are transiently excreted, secreted or otherwise handled abnormally.
3. Cancerous transformation of tissue results in the exudation of intracellular enzymes during abnormal cellular growth in the absence of necrosis.

To this may be added that necrosis is a common associated finding in malignant tissue, thereby affecting an already raised SGOT level.

The technique used for determining SGOT levels in this project was that of Karmen, Wroblewski and La Due⁽⁶³⁾ , and was carried out, as were all the biochemical investigations, in the laboratories of the McEachern Cancer Research Laboratory, at the University of Alberta.

CHAPTER 1, PART C.

BIOCHEMICAL INVESTIGATIONS

A series of biochemical investigations were carried out on the dogs at intervals during the induction of cirrhosis. These investigations were used as a form of "Biochemical Biopsy" as suggested by Wroblewski in his monograph on "Biochemical Biopsy via Body Fluids," so that some idea could be obtained concerning the state of liver function in particular and clinical health generally. Wroblewski suggests that the morphologic flora of tissues may have a counterpart in their enzymatic flora and the biochemical composition may at times be as characteristic of tissue as their cellular architecture. Tissue insults respond both by a morphologic change and enzymatic alterations in the blood. The tests used in this project were chosen so that they would represent a biochemical biopsy of the cirrhotic process at any particular time. They would, in fact, indicate the state of hepatic insufficiency at a particular time, which could be correlated with the SGOT level as representing the degree of hepatic necrosis present. The following biochemical determinations were carried out:

1. Serum Cholesterol (total), measured as milligrams percent. (64)

2. Serum Proteins (Total, Albumin, Globulin, and A/G ratio), measured as grams percent. (65)
3. Serum Alkaline Phosphatase measured as King-Armstrong units. (66)
4. Serum Bilirubin (total), measured as milligrams percent. (67)
5. Cephalin Cholesterol Flocculation, measured as 1+ to 4+ where 4+ represented the maximum. (68)
6. Bromsulphthalein retention test, measured as percentage retention. (69)
7. Blood Urea Nitrogen, measured as milligrams percent. (70)

The Blood Urea Nitrogen estimation was used both as an indication of general health as well as protein metabolism. Since carbon tetrachloride is known to produce some pathologic effect during its excretion by the kidneys, it was also used to interpret the state of renal function in the dog. Since cirrhosis is associated with impaired protein synthesis, this may be evidenced by a raised blood urea nitrogen level late in the disease.

Hemoglobin and Hematocrit estimations were also performed as an indication of general clinical status.

Hepatic insufficiency is a complex subject and no single hepatic function test is indicative of the capacity of the liver. The liver has numerous physiological activities and only a rough correlation

exists between the morphological and biochemical alterations which occur during diseases of this organ.

However, it is felt that the combination of tests used in this project is sufficient to give a fairly accurate estimate of hepatic function and some of its aspects in particular.

Abnormalities in bile secretion occur in cirrhosis leading to an increased total Serum Bilirubin. This may reflect either hepatocellular damage, obstruction of the intrahepatic or extrahepatic bile ducts, or increased erythrocyte hemolysis as occurs in secondary hypersplenism. In carbon tetrachloride poisoning, the changes in Serum Bilirubin levels are due mainly to hepatocellular damage.

The Bromsulphthalein retention test is an important test of the liver's function as an organ of excretion. This function becomes impaired in cirrhosis even when a minor grade of hepatic insufficiency exists, but there is only a fair correlation with morphological damage of the liver,^(71,72) and none with evidences of portal hypertension or ascites. However, Zieve and Hill, in 1955, found that this was the most reliable hepatic function test in the detection of cirrhosis.⁽⁷³⁾

Among the more important functions of the liver is synthesis of protein which may be measured in

numerous ways. For this project the estimation of Serum Proteins and Cephalin Cholesterol Flocculation were chosen as representatives of this group. Cirrhosis may be reflected by abnormalities of the various protein fractions; e.g., hypoalbuminemia, hyperglobulinemia, and by an increase in Cephalin Cholesterol Flocculation which is positive in nearly 90% of cases of hepatitis and from 50 to 80% of cases of cirrhosis.

The liver plays an important role in the metabolism of Cholesterol and hepatic insufficiency occurring in cirrhosis may reflect itself in a reduced Serum Cholesterol level. It may be raised in biliary cirrhosis due to obstruction of its excretion.

Enzyme metabolism of the liver has been gauged by the use of Serum Alkaline Phosphatase and SGOT estimations. The former is usually moderately raised in "active" cirrhosis. Any form of obstruction, either intrahepatic or extrahepatic will raise it markedly. As mentioned earlier, the SGOT estimation appears to be highly specific for hepatic insufficiency due to hepatocellular necrosis. These tests may be particularly valuable in following the clinical course of active cirrhosis.

CHAPTER I, PART D.

FURTHER EVALUATION

From the time the experiment started the general state of health of the animals was observed. Particular attention was paid to the following general aspects of their progress:

1. General alertness.
2. General physical condition.
3. Appetite.
4. Weight.
5. Abdominal examination for ascites.

1. This was judged by the reaction of the animal to feeding, being shown attention, the interest taken in surroundings and behaviour on short walks taken when brought up for weighing and blood testing.

2. This was judged by general appearance, sheen of coat, feel of musculature and liveliness on being walked. Evidence of weight loss, such as slack skin, increasingly obvious thoracic skeleton, was also noted.

3. The appetite was discerned simply by the quantity of food not eaten and the manner in which the dogs attended to their feeding.

4. The dogs were weighed at the regular intervals when blood was taken for biochemical testing.

This was especially necessary before performing the Bromsulphthalein retention test for the quantity of the drug injected is dependent on the animal's weight.

5. This examination consisted of the usual clinical approach to elicit ascites; e.g., observation, palpation, and percussion.

CHAPTER 1, PART E.

LAPAROTOMY AND LIVER BIOPSY

Each of the 10 dogs underwent a laparotomy under general anaesthesia at some stage during a period of three to five months following the institution of carbon tetrachloride poisoning as a means of producing hepatic cirrhosis. The following procedure was adhered to in each case.

1. Anaesthetic. Chloralose was used intravenously in the following dosage and manner. A 1% chloralose solution in 0.6% sodium chloride was prepared and maintained at a lukewarm temperature to prevent crystallization. Each dog was given 10 ml. of chloralose solution per kilogram body weight, via the intravenous route. In all cases one dose sufficed for full operative anaesthesia.

2. Position. The dog was positioned on the operating table on its back with all four limbs secured in extension.

3. Preparation. The entire abdomen and lower chest anteriorly was shaved and painted with iodine solution. The left thigh was shaved and used as the site of application of the ground electrode of the diathermy apparatus.

4. Incision. Midline, supra-umbilical, from the xiphisternum to umbilicus.

5. Procedure. Under sterile and aseptic conditions, the peritoneal cavity was opened through an upper midline abdominal incision. Bleeding, which was minimal because of the bloodless field through the "linea alba", was arrested with hemostatic forceps and then coagulated using the diathermy apparatus. Larger bleeders were ligated with double "0" cotton thread.

On entry into the peritoneal cavity, care was taken to leave the falciform ligament in position. The entire contents of the peritoneal cavity were then examined, special notice being taken of, (1) liver, (2) spleen, (3) presence of peritoneal fluid, (4) portal vasculature, (5) collateral circulation, (6) extra-hepatic biliary apparatus.

The portal pressure was then determined using a simple manometer filled with heparinized saline to prevent intraluminal clotting. (See Figure 1).

A portion of the jejunum was delivered from the peritoneal cavity and laid on the anterior abdominal wall. A suitable jejunal mesenteric vein was selected and the needle attached to the manometer tubing, inserted into it. The base of the manometer was then positioned at the level of the "porta hepatis" and the level of saline in the manometer allowed to settle at the height determined by the portal pressure. This was re-checked

by raising the manometer, allowing the fluid to thereby fall further and then replacing it at the level of the "porta hepatis" again so that the saline would climb to its former position thus ensuring the accuracy of the first reading. On removal of the needle from the jejunal mesenteric vein, the point of entry and the exit of the needle was occluded by a hemostat and ligated with cotton thread.

Following this procedure, a small wedge biopsy of the left lobe of the liver was performed in the following manner. Two interrupted atraumatic double "0" catgut sutures were placed near the edge of the left lobe of the liver, the one proximal to the liver's edge, the other placed about three-quarters of an inch more distally. The sutures entered the superior surface of the liver, came out on the inferior surface and were then passed from inferiorly through the liver substance to emerge finally on the superior surface of the organ again. A V-shaped wedge, apex pointing to the diaphragm, was then excised, after which the two sutures were firmly ligated approximating the incised edges of the liver and thereby obtaining hemostasis. This was usually sufficient to obtain adequate hemostasis, but in those instances where further hemorrhage ensued

another suture was placed. During the operative procedure each animal received 500 milliliters of 5% Dextrose in Water intravenously.

6. Closure. The peritoneum was closed with a continuous layer of "0" chromic catgut, while the "linea alba" was reapproximated using a continuous "0" linen thread suture. The skin was closed with a continuous "0" chromic catgut suture. No drains were used.

The animals were removed to their cages where they were allowed to recover.

The liver biopsy specimens were stored in formaldehyde and later sectioned and stained for (a) general cellular appearance, (b) reticulum. The following staining techniques were used:

- (1) Hematoxylin and Eosin. (74)
- (2) Reticulum. (75)

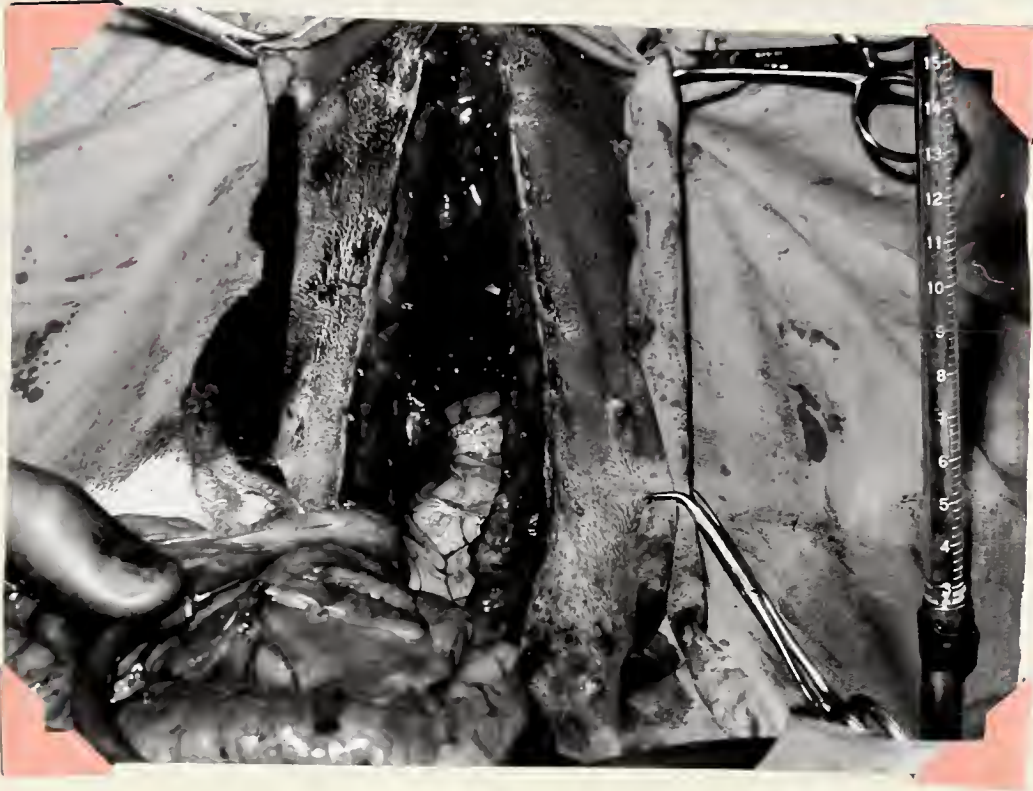


Figure 1.

CHAPTER II

PRESENTATION OF RESULTS

Portal cirrhosis of varying degree was produced in all ten dogs during a period of three to five months administration of carbon tetrachloride orally. The diagnosis was made on histological examination of biopsy specimens of the liver taken at laparotomy. In eight of the animals in which the portal pressure was measured it was raised in every instance and markedly so in several. Compared with the average results of twenty-six normal dogs used as the control, the average portal pressure of the experimental dogs was significantly raised statistically. Three of the dogs developed ascites but no evidence of an increased portal systemic collateral circulation was observed. (The latter observation was made simply by visual examination of the contents of the peritoneal cavity. The esophagus was not examined, nor were portal or splenic venograms performed.)

Liver function tests demonstrated progressive hepatic dysfunction. The controls for all the parameters of liver function used were calculated from the findings on twenty-six normal dogs.

Refer to Table #2 for comparison of results

of liver function and other hematological tests compared with the average results on the ten experimental dogs at the time of laparotomy.

The declining clinical status was noted by a gradual decrease in weight in most instances and the presence of ascites in three dogs, in two of which the weight was increased presumably due to the presence of intraperitoneal fluid.

At laparotomy the livers of all the dogs showed macroscopic evidence of cirrhosis, though the liver of dog K-25 appeared only slightly cirrhotic.

Cirrhosis was recognized macroscopically by a tawny to orange discoloration of the liver due to numerous small nodular areas of this color. The liver substance was nodular in appearance and on palpation. The consistency was firm to rubbery.

The results obtained in each dog will be presented and discussed separately following which the average results obtained in each dog over the experimental period are presented. A Table (#1) is presented demonstrating the key to the units used in all the investigations undertaken.

Preceding the presentation of results, microphotographs of normal dog liver are shown depicting the following techniques of staining:

- (1) Hematoxylin and eosin staining at magnifications of x 45, x 90, x 225.
- (2) Reticulum staining at magnifications of x 90.

These microphotographs are presented for comparison with the microphotographs shown depicting the varying stages and degrees of cirrhosis produced in the experimental animals.

However, before presenting the results of this project, a brief resume of the Pathological Aspects of Cirrhosis is outlined, as a basis on which to study and compare the histopathological results obtained.

DESCRIPTION OF THE STATISTICAL ANALYSIS

The averages, where mentioned, represent the arithmetic means: The standard deviation was obtained using the conventional formula:

$$s.d. = \sqrt{\frac{\sum x^2}{N} - \frac{(\sum x)^2}{N^2}}$$

Where $\sum x$ = the sum of all values.

N = the number of values

$\sum x^2$ = the sum of the squares of all values.

$(\sum x)^2$ = the square of the sum of all values.

s.d. = standard deviation.

The student's - "T" test was applied for statistical significance where possible.

PATHOLOGICAL ASPECTS OF CIRRHOSIS

Cirrhosis is a most important disease, particularly in the adult where it is one of the most frequent causes of death. An attempt will be made, before discussing the histopathological findings in the experimental animals, to survey briefly the pathophysiologic phenomena which characterize cirrhosis.

No one definition is as yet fully accepted but the one arrived at during the 1931 Proceedings of the International Society for Geographic Pathology in Geneva, seems to be most adequate. This definition includes an alteration in the normal hepatic architecture in which the presence of hepatocellular regeneration is associated with an increase in connective tissue. Microscopic examination is expected further to demonstrate hepatocellular degeneration, necrosis and the signs of chronic inflammation.

In our present state of knowledge it is not yet understood whether cirrhosis is a uniform process or develops over several different pathways -- whether it is a primary disturbance of hepatic cells with reactive tissue formation (7,8,9) or a primary mesenchymal lesion, inflammatory in character, in which the epithelial alterations are secondary to the

mesenchymal changes, particularly to the scarring. (23,24)

Functionally cirrhosis is characterized by:

- (1) Reduced hepatic function.
- (2) Portal hypertension.
- (3) Ascites.
- (4) Tendency to progression.

Briefly, the pathophysiologic features found in cirrhosis, will be discussed.

1. Hepatocellular degeneration and necrosis.

The etiologic factors in cirrhosis may produce.

a) Homogeneous cytoplasmic coagulation and single cell necrosis. This is seen particularly in cirrhosis developing from hepatitis. It results in an accumulation of mononuclear cells and collapse of the framework. Not usually seen in the other types of cirrhosis.

b) Focal granular clumping of the cytoplasm, which is usually seen in swollen cells. The clumps aggregate to form a ramified perinuclear acidophilic body, called by Mallory⁽²⁴⁾ "alcoholic hyaline", and presumably a specific effect of alcoholic intake.

c) Centrolobular necrosis, which occurs mainly in chemical poisoning, toxic hepatic injuries and congestion. Cirrhosis itself, by virtue of its mechanical effects on the flow of blood and bile may produce -

(a) Ischemic degeneration and necrosis, which usually develops in the centre of the nodule resembling

toxic or congestive necrosis, indicating that the underlying cause is probably oxygen deficiency interfering with enzymatic processes.

(b) Topical effects of bile on the hepatic cells, resulting in bile pigmentation of hepatic cytoplasm, with subsequent fibrosis, interrupting communication between liver cells and bile ductules.

2. Massive necrosis, which may be due to the original cause of cirrhosis itself, may be followed by regeneration or collapse of the cellular framework which produces stress resulting in break fissures in the surrounding parenchyma.

3. Fatty metamorphosis may be due to the original cause and therefore precede the cirrhosis, or result from the mechanical changes. Seen particularly in alcoholism, malnutrition and various deficiencies - e.g. choline, protein. The major alteration chemically is a decrease in the DPN content of the cells. A tendency to fibrosis occurs.

4. Inflammatory reaction and Splenomegaly

Inflammation may be due to the original cause of the cirrhosis or secondary to hepatocellular necrosis.

The response is probably mainly due to the liver cellular injury. The cells consist mainly of mononuclear leukocytes, but polymorphonuclear leukocytes,

giant cells and histiocytes are seen in increased numbers as well. The splenomegaly is probably due to a combination of portal hypertension and reactive hyperplasia.

5. Regeneration. This process takes place normally but at an unknown rate, which is increased wherever hepatic necrosis occurs. The precise form of stimulation of regeneration is also not known. The interruption of the liver cell plates is thought to be the stimulus. Another hypothesis involves the removal of a growth inhibiting factor, suggesting a humoral control of both normal and abnormal regeneration. Histologically, there is noted an increased cytoplasmic basophilia, nuclear and nucleolar enlargement, multiplication of cells, an increased number of multinuclear giant cells and the formation of liver cell plates more than one cell thick. Regeneration occurs mainly in the periportal zone, and accentuation of this process in circumscribed areas results in the formation of nodules, with resultant pressure on surrounding stroma and parenchyma producing fibrous tissue septa. Sinusoids may merge at the centres of lobules forming central veins, and with the growth of portal vein branches and bile ductules into them, secondary "lobulization" occurs.

6. Ductular cell reaction. In cirrhosis there is an excessive proliferation of ductular cells, and ductules,

singly or in clumps. This, too, is interpreted as a response to cellular necrosis. The proliferation is noted frequently in connective tissue trabeculae and the portal tract. Whatever its origin, the ductular cell reaction is a response of the liver to injury of various kinds. There is also usually a periductular fibroblastic reaction resulting in periductular fibrosis.

7. Fibrosis.

Fibrosis is a common finding in cirrhosis, a feature which is necessary for its diagnosis, and one, thought by many workers, to be the most significant change that occurs in the liver in cirrhosis. It would seem justifiable therefore to discuss briefly the histogenesis and morphology of this entity. The hepatic connective tissue framework consists of reticulum fibers and collagenous fibers and membranes. In cirrhosis there is a marked increase in both substances which results from:

- (a) A collapse of preformed stroma.
- (b) New formation of connective tissue, and
- (c) A combination of both.

The collapse is a direct result of hepatocellular necrosis while the new formation of connective tissue is associated particularly with ductular cell proliferation, resulting in fibers radiating septally from the

portal tracts into the parenchyma. Fibroblasts are to be found whenever there is an increase in fibrosis. As a result of this increase in fibrosis by various factors and pathways, its morphology is varied. The centrolobular form is found mainly in congestive and toxic conditions of the liver. Focal intraparenchymal areas of fibrosis may occur, while involvement of the portal tracts, the most common site of fibrosis in cirrhosis, is particularly associated with pericholangitis and extrahepatic biliary obstruction. The periportal form extends into the adjacent parenchyma either as a diffuse widening or a stellate enlargement. The periductular form is probably due to the regurgitation of bile in intrahepatic and extrahepatic cholestasis. In septal fibrosis, bands of connective tissue traverse the parenchyma of the organ. Post collapse fibrosis, resulting from massive and submassive necrosis is characterized by broad bands of connective tissue.

8. Changes in the hepatic vasculature. These have been studied mainly by injection techniques of isolated livers, and splenoportal radiography during life. Branches of the portal vein show deformities and an irregular arrangement, especially when associated with ascites. The arteries appear normal, while the hepatic vein tributaries, not protected by surrounding connective

tissue, are easily compressed both by fibrosis and regenerative nodules. This postsinusoidal compression interferes with the drainage of blood from the liver and is one of the main causes of portal hypertension in cirrhosis.^(21,33,34) Smaller nodules compress veins more effectively than larger ones and are associated with a greater tendency to portal hypertension.⁽¹⁵⁾ Parasinusoidal communications occur between branches of portal and hepatic veins, which shunt blood from portal into hepatic veins, bypassing the parenchyma. Together with extrahepatic portosystemic and porto-pulmonary shunts,⁽³⁸⁾ they divert blood from the hepatic parenchyma. Presinusoidal anastomoses between portal venous and hepatic arterial branches also occur in cirrhotic livers, and in this manner contribute to the portal hypertension. It seems likely, therefore, that the total effect on the hepatic circulation is the resultant of the various hemodynamic forces acting on it, and helps to explain why there is no proportionate relationship between the type and degree of cirrhosis, the level of portal pressure and the presence or absence of ascites. The important functional aspects associated with cirrhosis of the liver are:

1. Portal hypertension.
2. Reduced liver function.
3. Ascites.

Portal hypertension results mainly from regenerative nodules causing postsinusoidal obstruction, vascular anastomoses especially presinusoidal between the hepatic arterial and portal venous circulation, and fibrosis producing distortion of the hepatic and portal veins.

Reduced liver function is due mainly to parenchymal cellular damage and diminution of the hepatic blood supply which accrues from mechanical obstruction to the flow of blood and the shunting of blood from the portal to the systemic circulation by intrahepatic and extrahepatic portosystemic anastomoses.

Ascites appears to be due to a number of factors, the relative importance of which have not yet been elucidated. The increased hydrostatic pressure present in portal hypertension encourages the transvascular flow of fluid to the peritoneal cavity. There is also an increased formation of hepatic lymph resulting in its escape into the peritoneal cavity. The disturbance of hormonal inactivation, especially aldosterone, by a damaged liver is said to participate in the formation of ascitic fluid while, finally, the depletion of plasma proteins reduces the osmotic pressure of the blood, further tending to facilitate the transvascular flow of fluid to the peritoneal cavity.

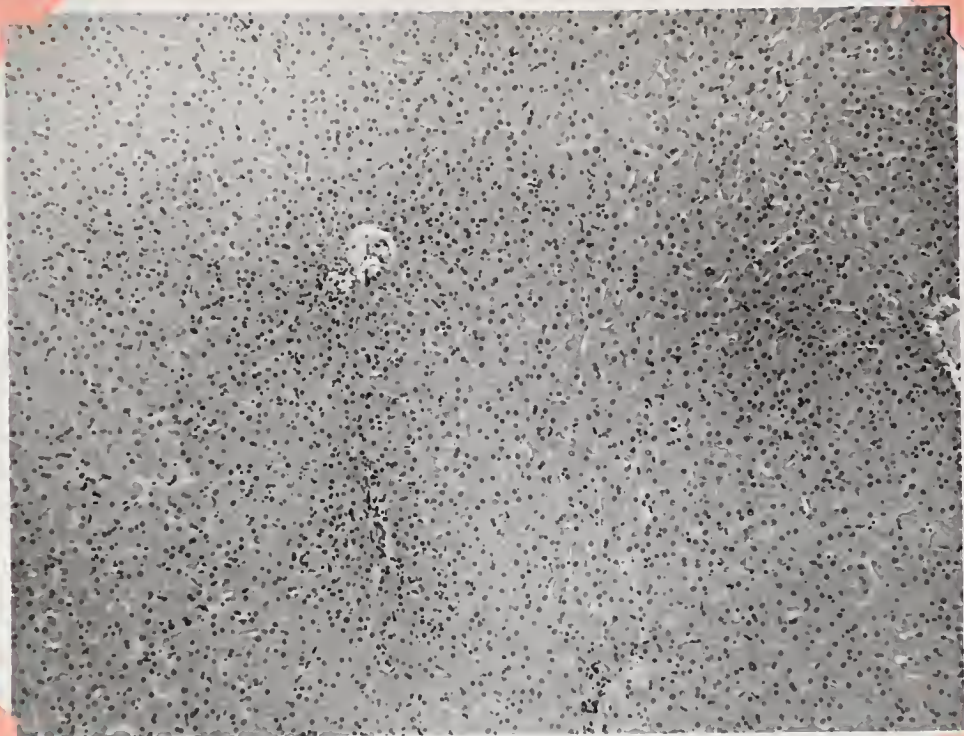


Fig. 2

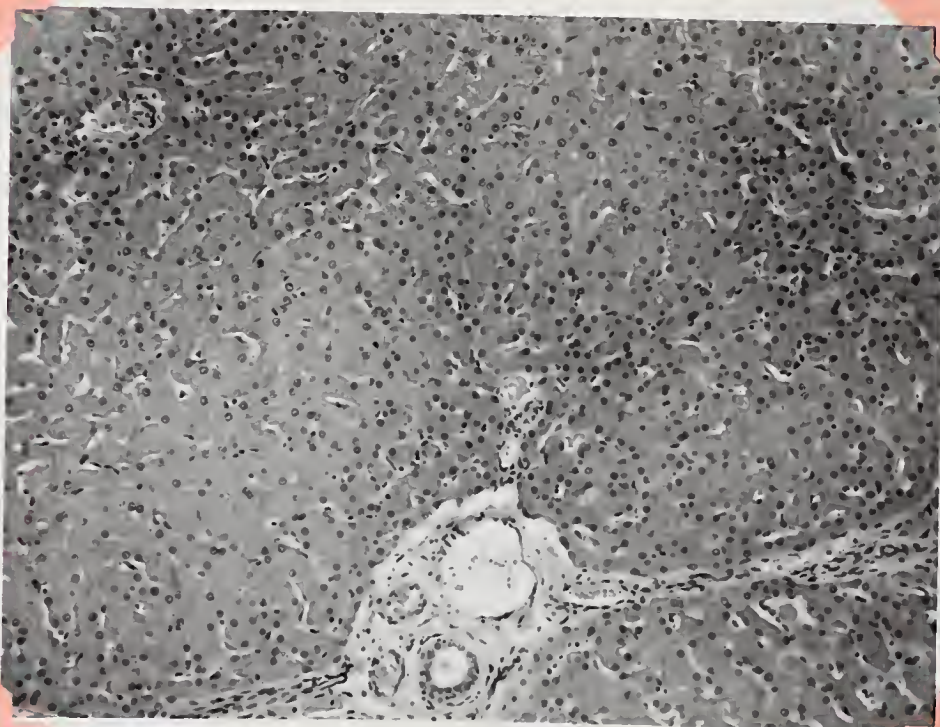


Fig. 3

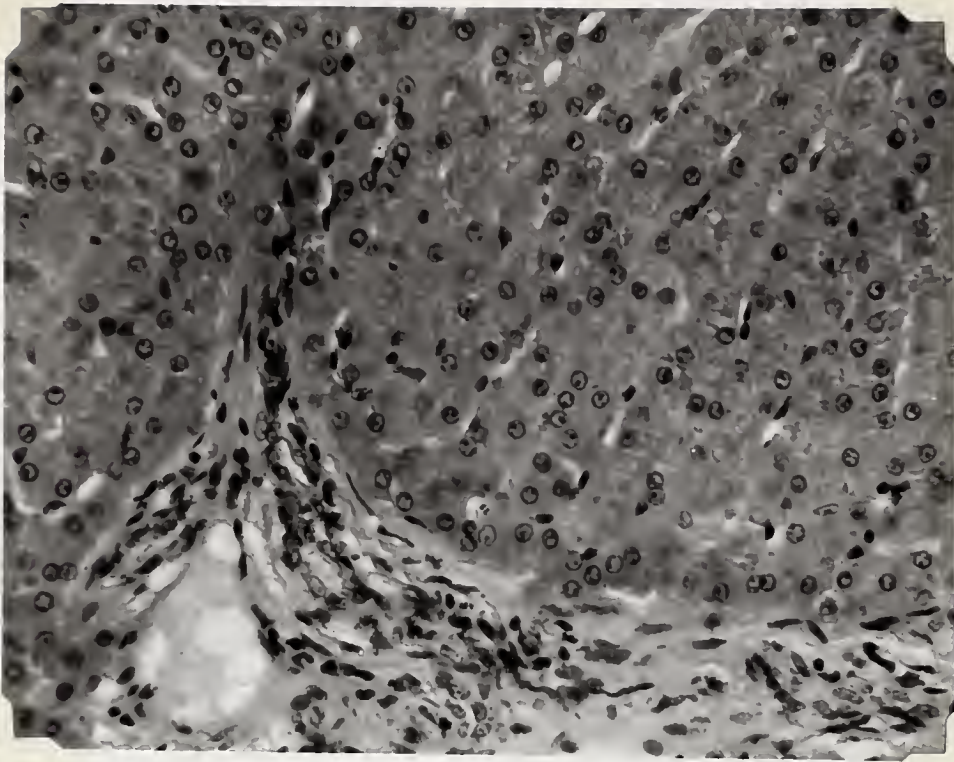


Fig. 4

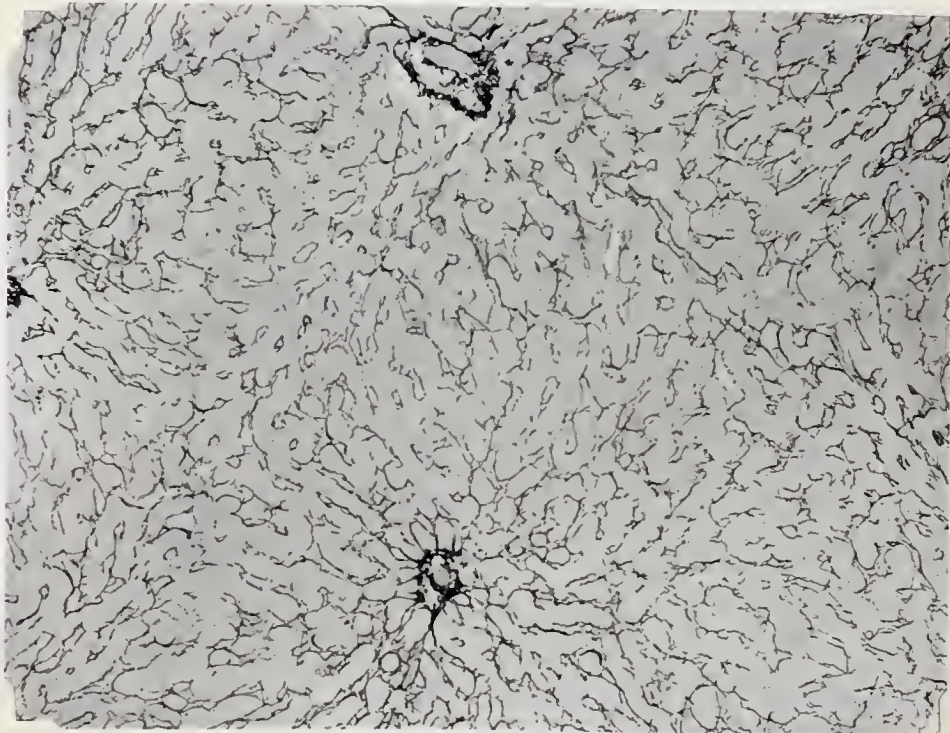


Fig. 5

KEY TO UNITS USED IN INVESTIGATIONS

1. SERUM PROTEINS - gm %
2. SERUM ALKALINE PHOSPHATASE - King Armstrong Units
3. SERUM CHOLESTEROL - mgm %
4. SERUM BILIRUBIN - mgm %
5. SERUM GLUTAMIC OXALACETIC TRANSAMINASE - Units/min.
6. BROMSULPHTHALEIN TEST - % Retention
7. BLOOD UREA NITROGEN - mgm %
8. CEPHALIN CHOLESTEROL TEST - + to ++++ Units
9. HAEMOGLOBIN - gm %
10. HAEMATOCRIT - % Cells
11. MESENTERIC PRESSURE - mm Water

Table 1.

DOG K-25

Hematological and biochemical investigation on the 16th of November, 1959, revealed no abnormality of liver function. Clinically the animal was alert, fit and healthy. Oral feeding with carbon tetrachloride in gelatin capsules commenced on the 18th of November, 1959. Initially the animal was given 1.5 ml. carbon tetrachloride every second to third day. This was gradually increased until at the time of laparotomy, 138 days after the start of the experiment, 10 ml. of carbon tetrachloride was being administered on alternate days. The dose of carbon tetrachloride was regulated by the level of SGOT. The aim was to keep the SGOT level below 100 units so that gross hepatic necrosis would be prevented. Over the period of 138 days a total of 242.5 ml. carbon tetrachloride was administered. During this period the dog remained healthy and increased his weight from 14.75 kg. initially, to 17.5 kg. at the time of laparotomy.

Laparotomy was performed on the 4th of April, 1960, 138 days after this experiment began. The operation was carried out under aseptic conditions observing the usual operating room techniques.

Anaesthetic. Chloralose was administered by the intravenous route. 180 ml. of 1% chloralose solution in 0.6% sodium chloride solution was administered. It was necessary to inject the solution in a lukewarm state in order to prevent crystallization of the chloralose. The induction of anaesthesia and its maintenance during the surgical procedure was smooth and uneventful.

Incision. Midline.

Procedure. The peritoneal cavity was entered through a midline approach. Skin, subcutaneous tissue, linea alba and peritoneum were incised in that order. Hemostasis was obtained by ligation of the larger vessels and coagulation of the smaller bleeders. On opening the peritoneum a general examination of the peritoneal cavity was carried out, paying particular attention to the liver, spleen, the presence of ascitic fluid and evidence of an increased portal systemic collateral circulation. Following this the portal pressure was measured. This was accomplished by laying a segment of jejunum with its mesentery on the anterior abdominal wall and introducing a 20 gauge needle connected by a rubber tube to a simple manometer, into a vein in the mesentery. The needle, tube and manometer were filled with heparinized saline to prevent coagulation.

The lowest level of the manometer was then placed on a plane level with the porta hepatis, and the level of the saline in the manometer allowed to settle at the pressure within the mesenteric vein. In order to be satisfied that the manometer was not blocked and that the level was true the manometer was raised to allow the level of saline to descend further and then replaced at the original level and the column of saline in the manometer allowed to attain its former correct pressure level. After the height of the column of fluid had been measured indicating the pressure of portal blood in the mesenteric vein the needle was removed and the site of the punctured vein occluded with a hemostat and ligated with triple "O" catgut. The liver biopsy was then performed. Two double "O" chromic catgut sutures were placed, one proximal to the other, at the edge of the right portion of the middle lobe of the liver. The sutures passed from the ventral aspect to the liver through its whole thickness and then returned through the dorsal surface back to the ventral surface, a distance of 2 cm. separating the pathways of each suture track. A wedge shaped biopsy with its base at the liver edge was excised between the sutures which were then tautened and ligated to prevent bleeding from the incised liver surface. The biopsy specimen was

immediately stored in formaldehyde solution. The procedure was terminated by removal of any free blood from the peritoneal cavity and assurance that hemostasis was complete before closure was started.

Findings. The liver was normal or slightly enlarged. It was of a rubbery consistency exhibiting a pale, smooth, granular surface covered with small tawny colored areas measuring 1 - 2 mm. in diameter. The spleen appeared normal. There was no evidence of portal systemic collateral circulation, nor was ascites present. No other abnormality was noted. The portal pressure measured 119 mm. of saline.

Closure. The peritoneum was closed with a continuous layer of double "O" chromic catgut suture material. The linea alba was reapproximated by a continuous layer of "O" chromic catgut suture material using single "O" chromic catgut sutures intermittently as reinforcements. The skin was closed with a continuous single "O" chromic catgut suture. No form of drainage of the peritoneal cavity was used.

HISTOPATHOLOGY.

This specimen demonstrates a diffuse centrolobular, and in many regions peripheral lobular degeneration and necrosis. This has resulted in a distortion of the lobular pattern and its vascular arrangement.

Central veins and portal tracts are irregularly situated, either drawn nearer or further from each other by the irregular collapse of the cellular framework.

The reticulum stained specimen exhibits clearly the cellular collapse with the formation of reticulum membranes which traverse the liver structure dividing it up irregularly. The vessels, though irregularly arranged, remain patent. In some regions, the reticulum stain shows evidence of attempts at very early nodule formation.

Finger-like sheets of tissue are present throughout the specimen. It is difficult to assess their nature without special connective tissue stains, but they may either be new connective tissue (fibrosis) appearing in response to the hepatocellular injury, or compressed sheets of necrosed cells forming connective tissue-like membranes.

Beginning regeneration is noted with occasional attempts at nodule formation. Inflammatory cells, mainly monocytic in type, are present diffusely throughout the specimen. Occasional bile duct proliferation is also noted.

This specimen represents a liver which mainly shows evidence of fairly diffuse cellular degeneration and necrosis, with mild obscuration of the architecture. There appears to be a slight diffuse increase in

connective tissue and early attempts at regeneration. This specimen may possibly represent a very early stage in the genesis of cirrhosis, a pre-cirrhotic phase, following a phase of degeneration and necrosis. (See Figs. 6 - 9).

The absence of ascites and the only slight rise in portal blood pressure are in keeping with the above findings.

BIOCHEMISTRY

Several of the liver function tests appeared to bear a definite relationship to the level of SGOT, BSP retention, Serum Alkaline Phosphatase, and Serum Cholesterol were noted to fluctuate in a similar manner with the level of SGOT. The Transaminase level itself bore a proportional relationship with the quantity of carbon tetrachloride administered. The Serum Bilirubin level demonstrated a gradual increase with two maximum levels which corresponded with similar peaks in the SGOT levels. After the first 30 days the Serum Bilirubin remained above the average level of this parameter in the normal dog. Serum Alkaline Phosphatase, BSP retention and Serum Cholesterol also remained well above the average levels.

Cephalin cholesterol flocculation increased gradually throughout the period of the experiment, recording a measurement of 4+ at its termination. The

results of investigation of changes in Serum Proteins were as follows. There was a gradual but slight decrease in the Total Protein level. This was followed by a similar pattern with the Serum Albumin. Both fell well below normal average levels. Serum Globulin, on the other hand, dropped during the first month and then gradually rose to well above the average normal level. The Albumin-Globulin ratio after an initial rise during the first month gradually descended becoming reversed two weeks before the termination of the experiment.

Apart from an initial elevation, still within normal limits, the BUN remained unchanged. (See Figs. 10-20).

Hematology. The changes in Hemoglobin concentration and Hematocrit readings were not significant and remained more or less constant throughout the experiment, fluctuating only slightly within the limits of normality. (See Figs. 21,22).



Fig. 6

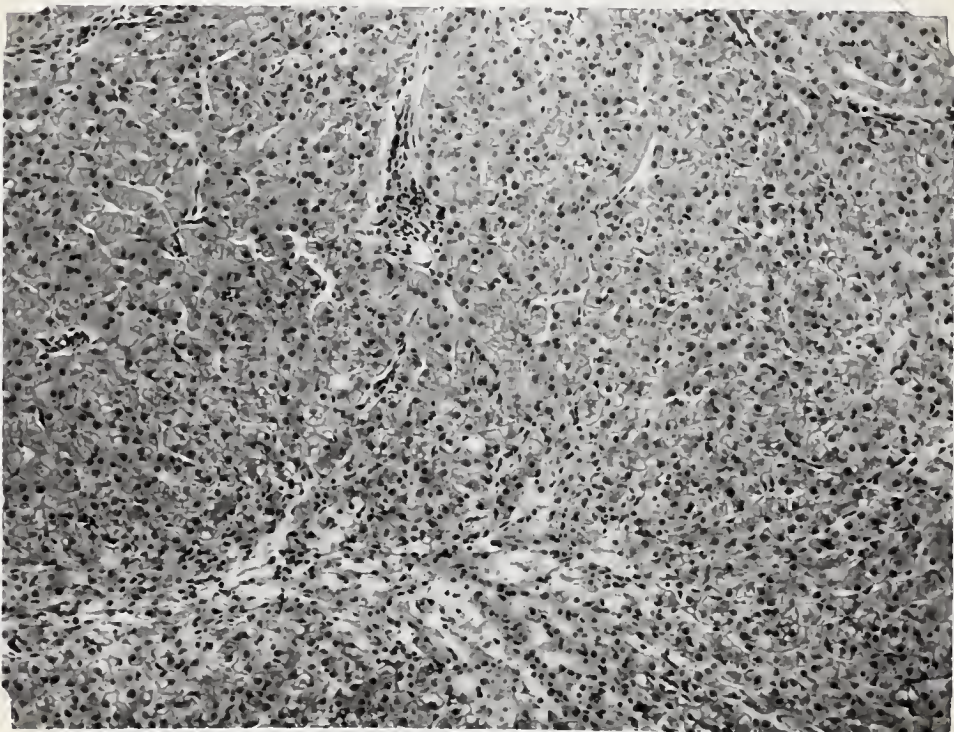


Fig. 7

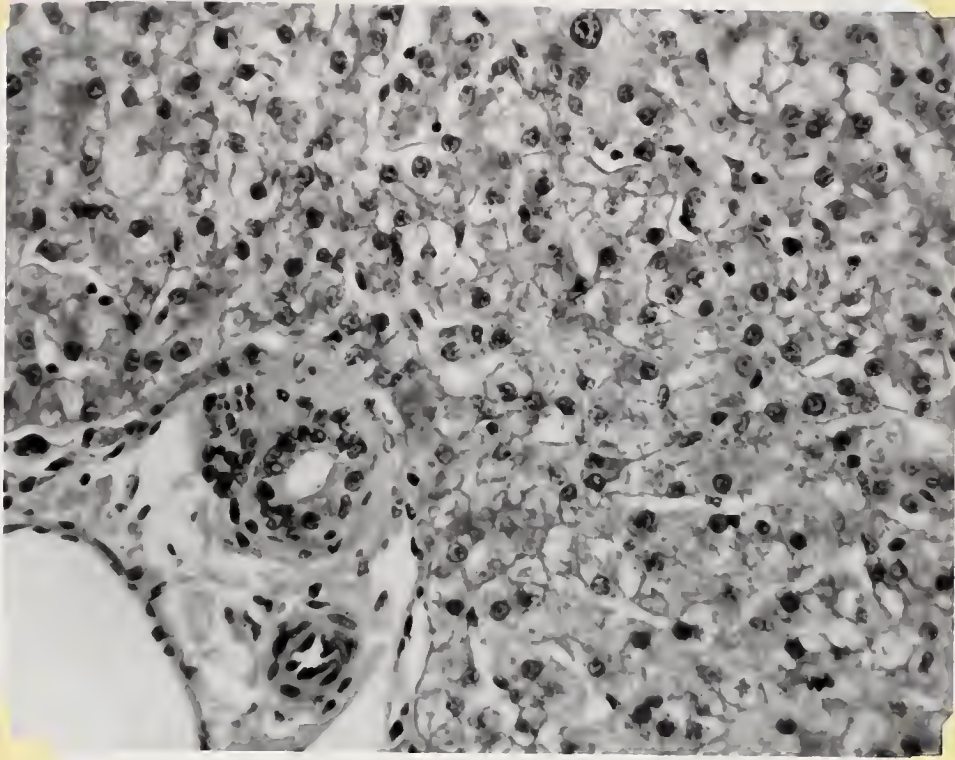


Fig. 8

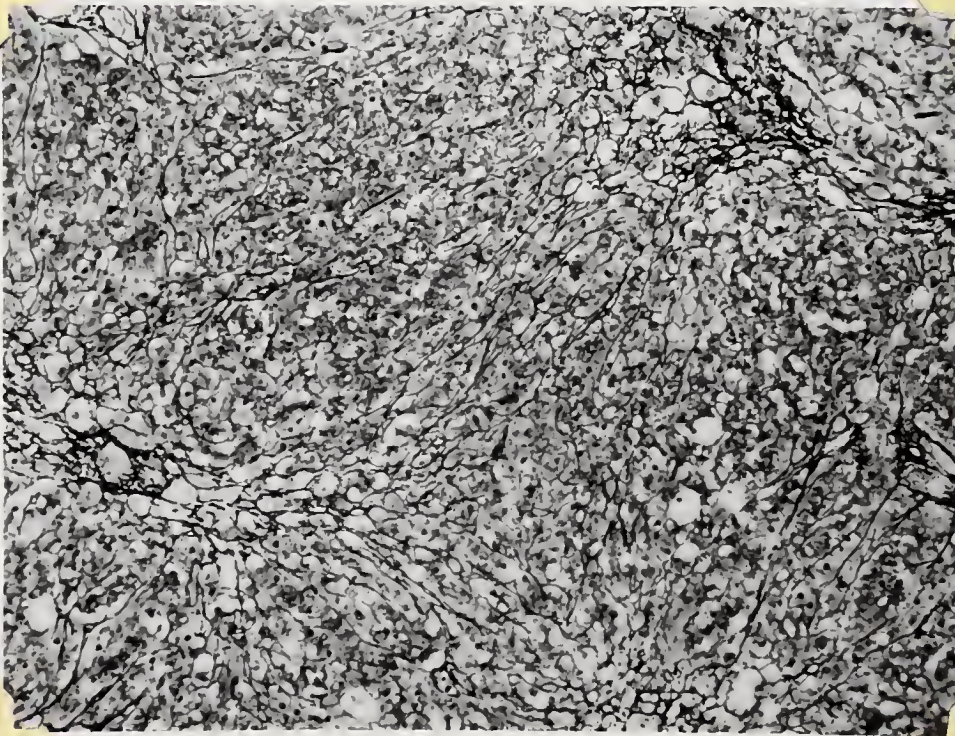


Fig. 9

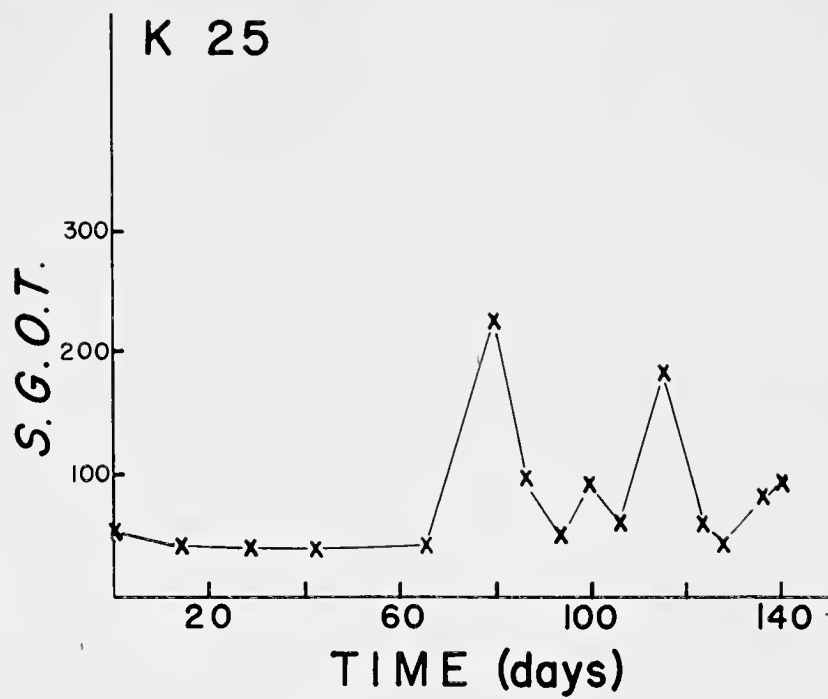


Fig. 10

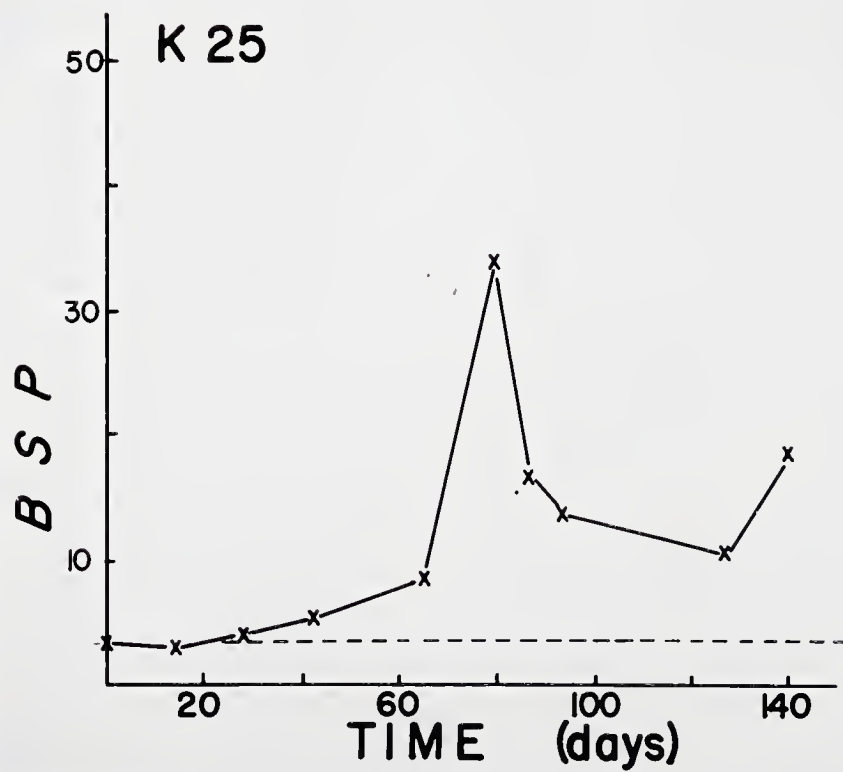


Fig. 11

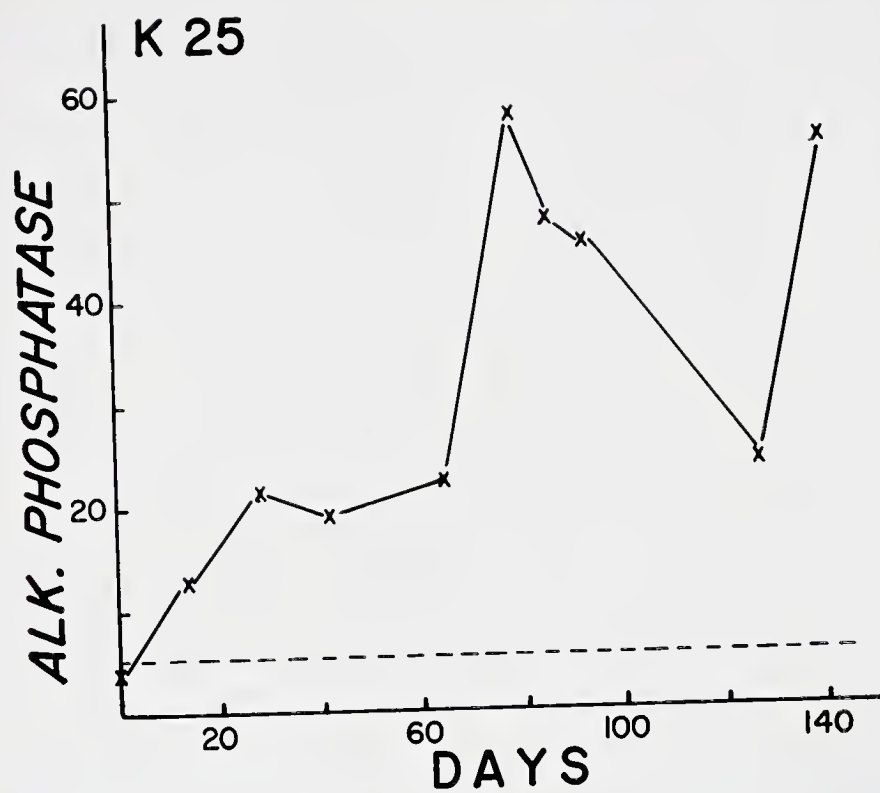


Fig. 12

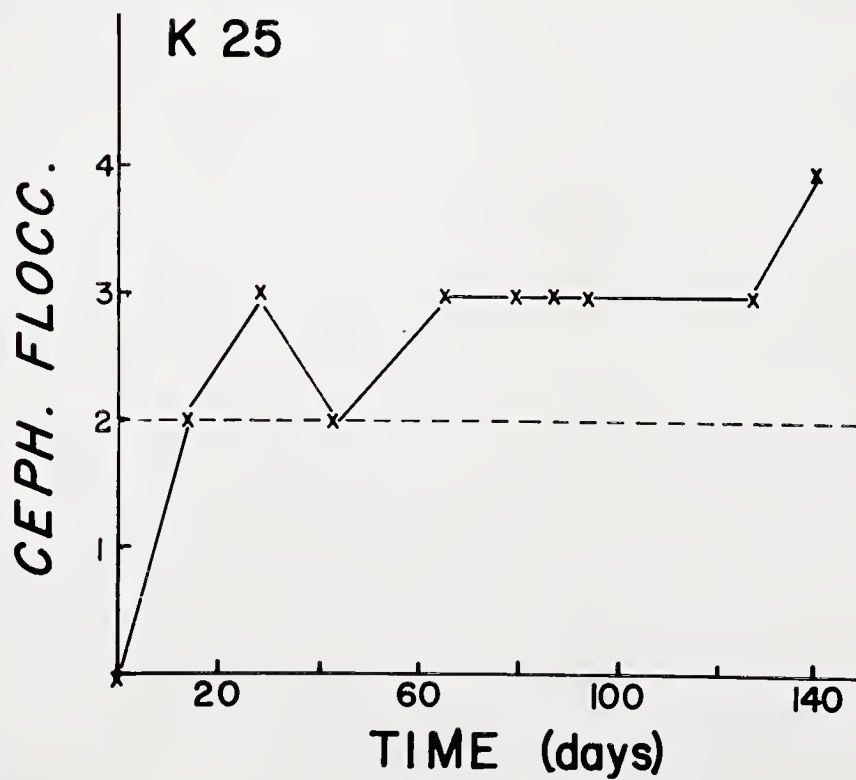


Fig. 13

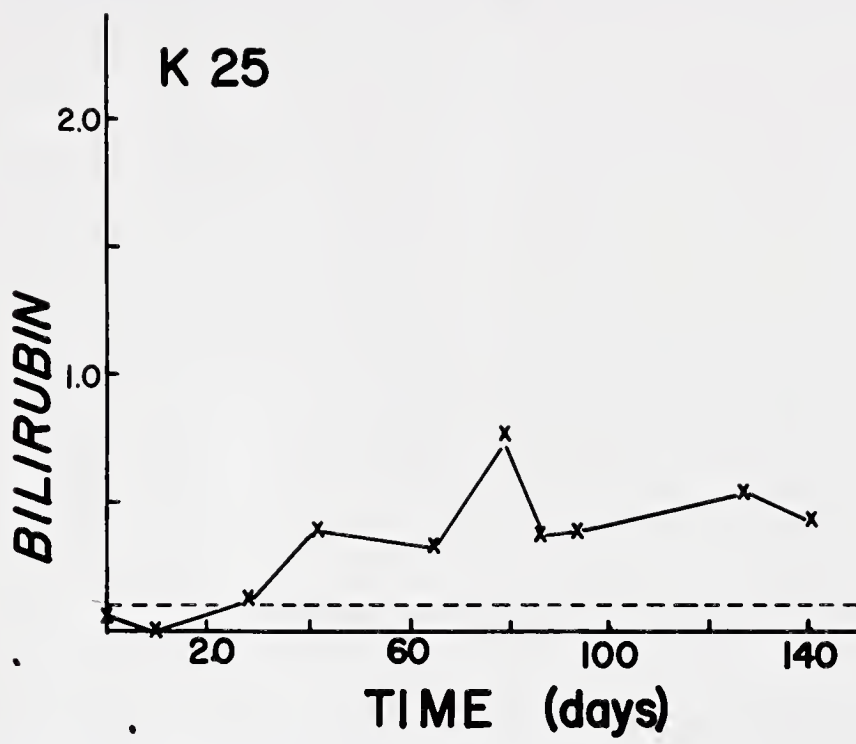


Fig. 14

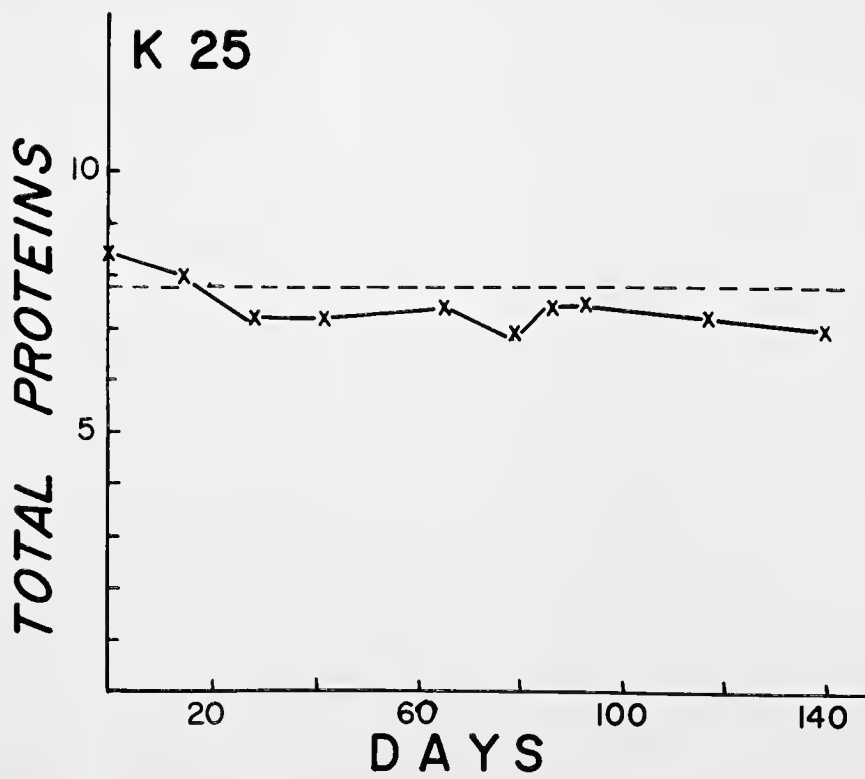


Fig. 15

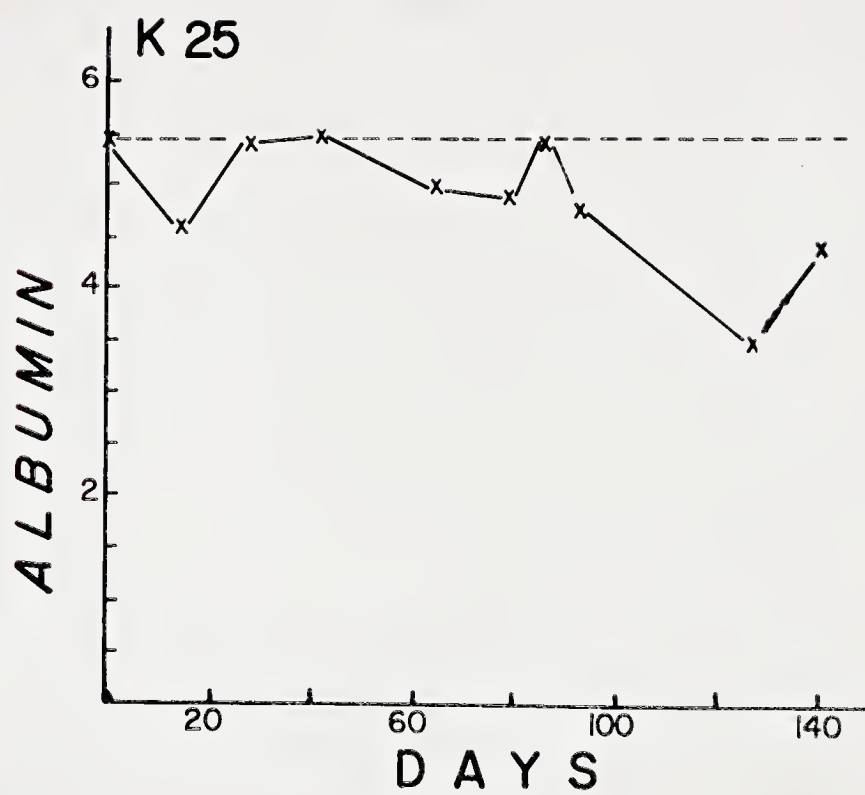


Fig. 16

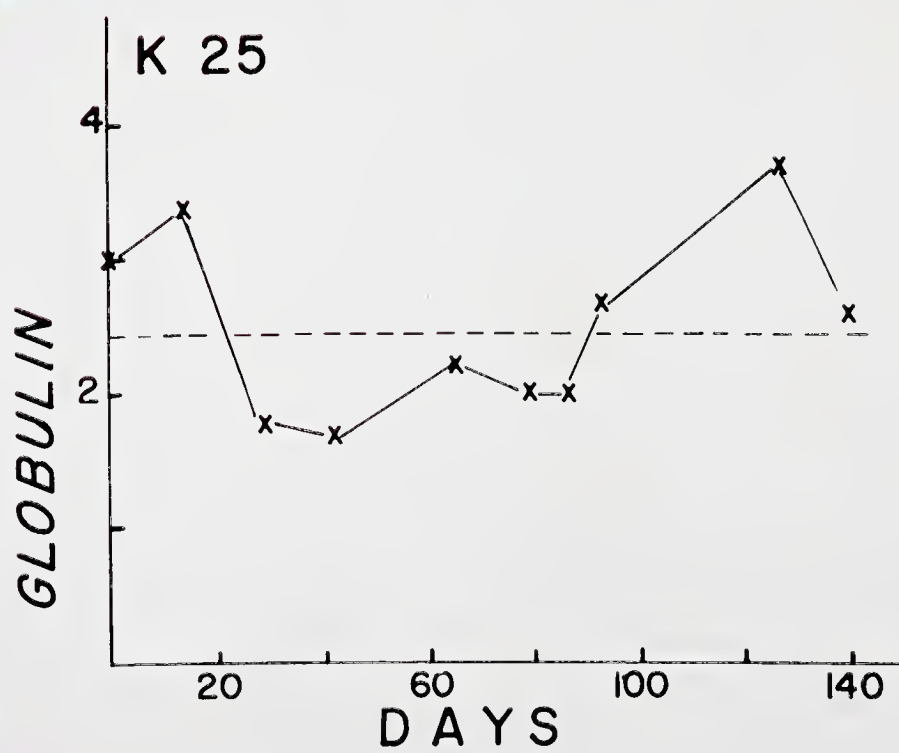


Fig. 17

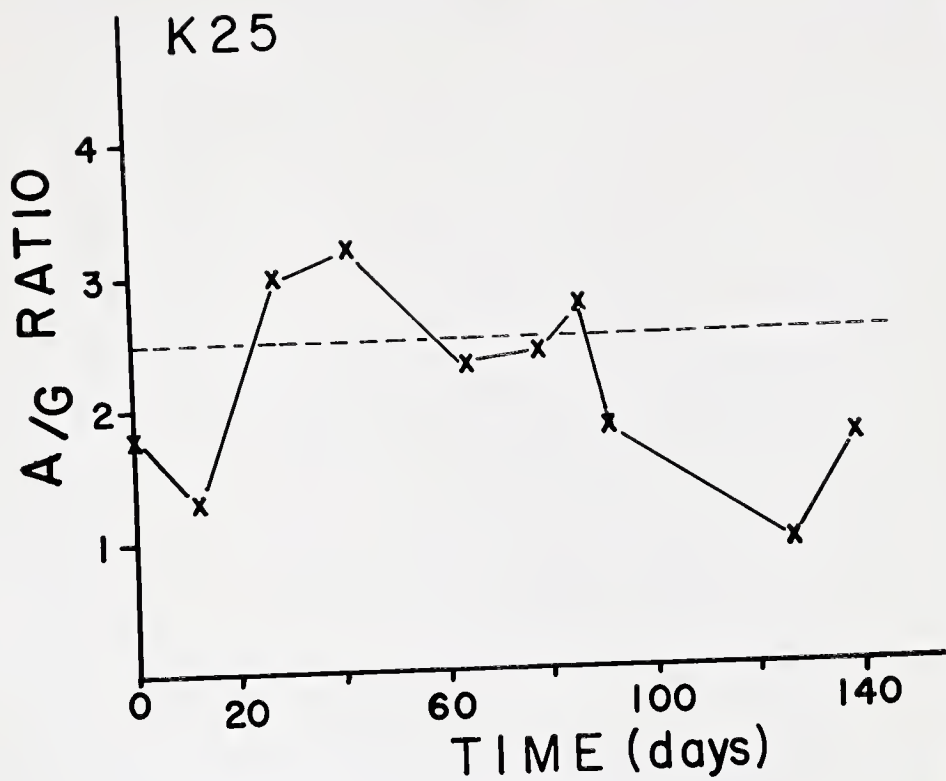


Fig. 18

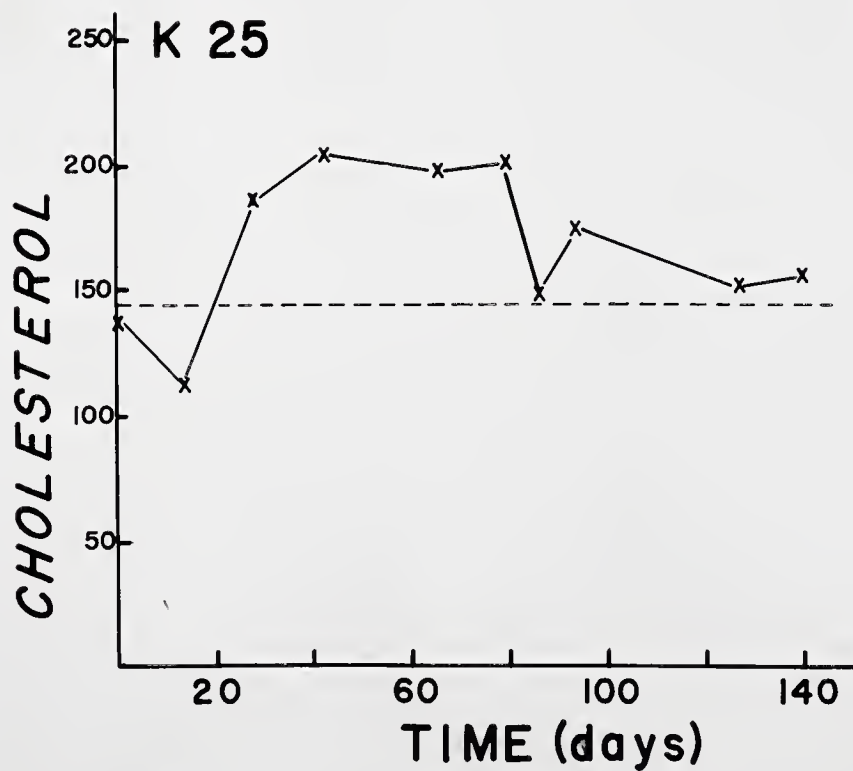


Fig. 19

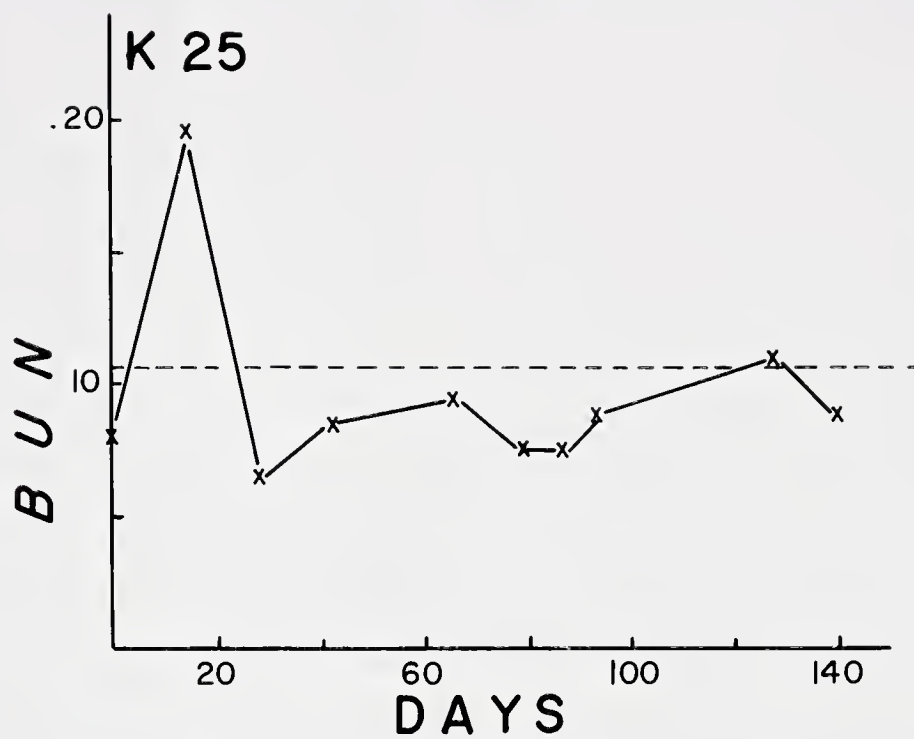


Fig. 20

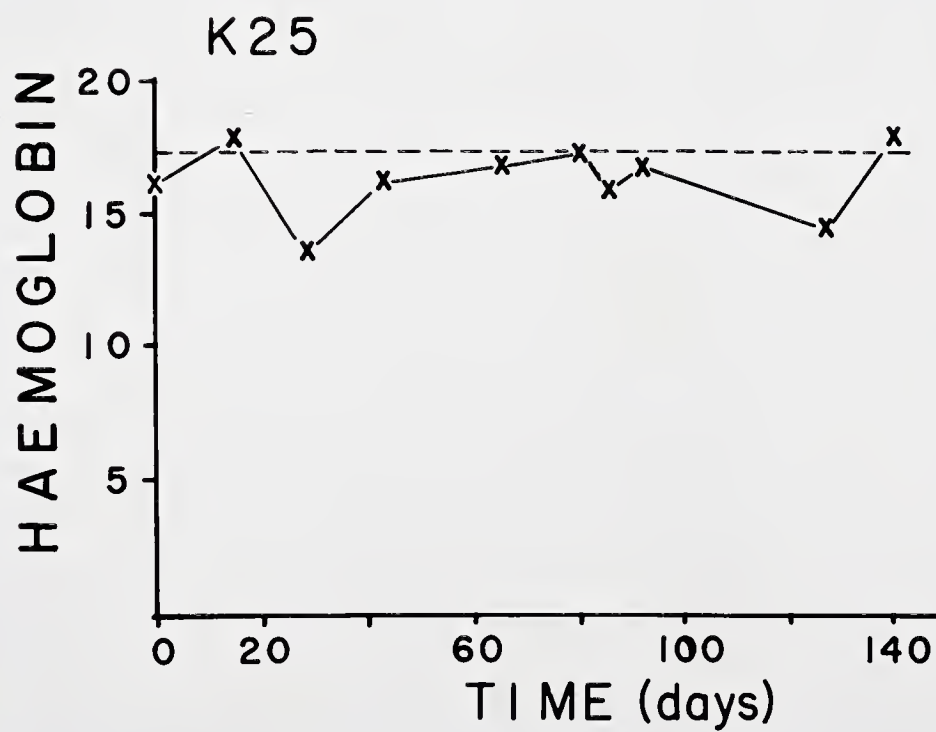


Fig. 21

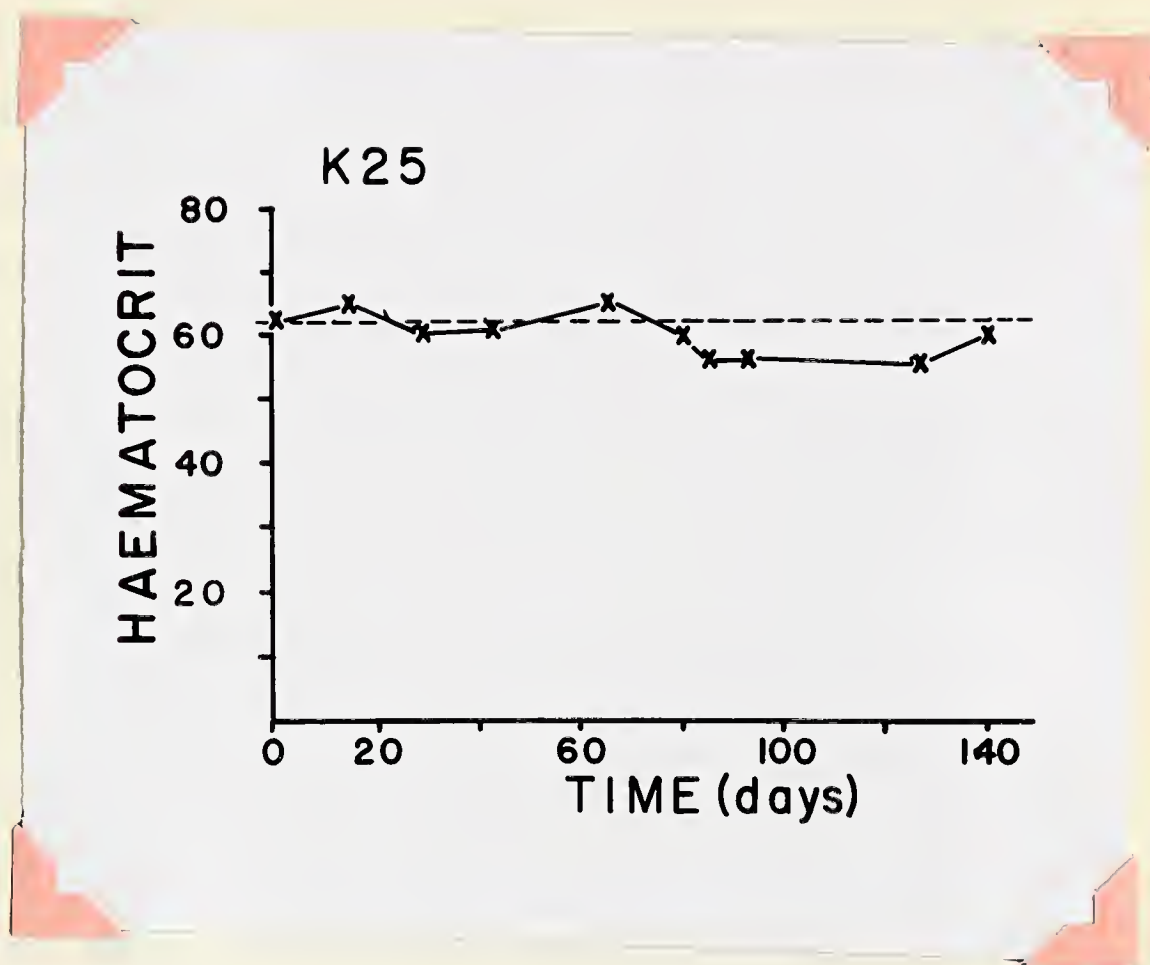


Fig. 22

DOG K-26

Hematological and biochemical investigation on the 16th of November, 1959, revealed normal liver function. Clinically the animal was alert, fit and healthy. Oral feeding with carbon tetrachloride in gelatin capsules commenced on the 18th of November, 1959. Initially the animal was given 1.5 ml. carbon tetrachloride every second to third day. This was gradually increased until at the time of laparotomy 138 days after the start of the experiment, 10 ml. of carbon tetrachloride was being administered on alternate days. The dose of carbon tetrachloride was regulated by the level of SGOT. The aim was to keep the SGOT level below 100 units so that gross hepatic necrosis would be prevented. Over the period of 138 days a total of 237 ml. carbon tetrachloride was administered. During this period the dog remained healthy although his weight decreased from 18 kg. initially to 16.25 kg. at the time of laparotomy.

For approximately two weeks prior to laparotomy the abdomen was noted to be distended. Clinical examination suggested the presence of ascites. Laparotomy was performed on the 4th of April, 1960, 138 days after the experiment began.

Anaesthetic, incision, procedure and closure were similar to that described for Dog K-25.

Findings. The liver was enlarged, congested, swollen and nodular. The consistency varied from firm to hard. Incisional biopsy confirmed the hard consistency which cut grittily in the manner usually ascribed to an unripe repair. The nodularity was fine to moderate. The spleen appeared normal and there was no evidence of a portal systemic collateral circulation. However, ascites was present in sufficient quantity for the intestines to float gently in it. The fluid was of a clear straw colored character. No other abnormalities were noted. The portal pressure measured 290 mm. of saline.

HISTOPATHOLOGY

The architecture of this specimen is so heavily obscured that normal liver architecture is absent. There is a diffuse increase in connective tissue which has so infiltrated the parenchyma that the lobular pattern is entirely lost. This diffusely irregular fibrosis is embedded on a background of hepatocellular degeneration and necrosis. Occasional isolated areas of hemorrhagic necrosis are present. In other areas groups of large sinusoidal-like structures, possibly central and hepatic veins thrown together by collapse and distortion of the

parenchyma are present. The fibrosis is so diffuse as to involve and enmesh all structures, being occasionally heavy in the portal tracts.

There is a generalized and diffuse bile duct proliferation, also seen particularly in the portal tracts.

The portal tracts themselves are distorted and irregular, presumably due to the disfiguring effect of active fibrosis and pressure produced by nodular regeneration.

This diffuse nodularity is well demonstrated by the reticulum stained specimens which also exhibit clearly the severe amount of necrosis that has taken place. This is shown by areas of tissue framework collapse varying in size from centrilobular to the multilobular zones of necrosis seen in the postnecrotic variety of cirrhosis.

Inflammatory cells mainly of the monocytic variety, giant cells and histiocytes are scattered diffusely throughout the tissue.

This specimen appears to demonstrate a mixed form of diffuse septal and postnecrotic cirrhosis, which has apparently been occurring simultaneously. It is not surprising that such a distortion of the hepatic architecture and its vascular arrangement should result in ascites and moderately severe portal hypertension. What is a little

unusual is the fact that such a necrotic process has been accompanied by a SGOT level which remained fairly stable below 70 units, except for a few occasions when it was elevated, but never to more than 120 - 170 units. (See Figs. 23 - 26).

BIOCHEMISTRY

Again, several of the liver function tests appeared to bear a definite relationship to the level of SGOT, BSP retention, Serum Alkaline Phosphatase, Serum Bilirubin and Serum Cephalin flocculation levels were directly proportional to the level of SGOT, which is interpreted as the degree of hepatic necrosis present at a given time. The BUN, on the other hand, bore an inversely proportional relationship to the SGOT level. There was a gradual decrease in the level of Serum Cholesterol as the experiment progressed.

The Total Serum Protein level fluctuated within the limits of normality, the final reading being over 1 gm.% lower than the level at the start of the experiment. Serum Albumin gradually decreased to well below the average normal level while the reverse occurred for the serum globulin level. The Albumin-Globulin ratio which bore an inversely proportional relationship to the level of SGOT gradually decreased to below the average normal level, being reversed at the time of laparotomy.

The level of Serum Alkaline Phosphatase

gradually rose to well above the average normal levels. This also occurred with Serum Bilirubin, BSP retention and Serum cephalin flocculation. (See Figs. 27 - 37).

Hematology. The changes in Hemoglobin concentration and Hematocrit readings were not significant and remained more or less constant throughout the experiment fluctuating only slightly within the limits of normality. (See Figs. 38,39)



Fig. 23

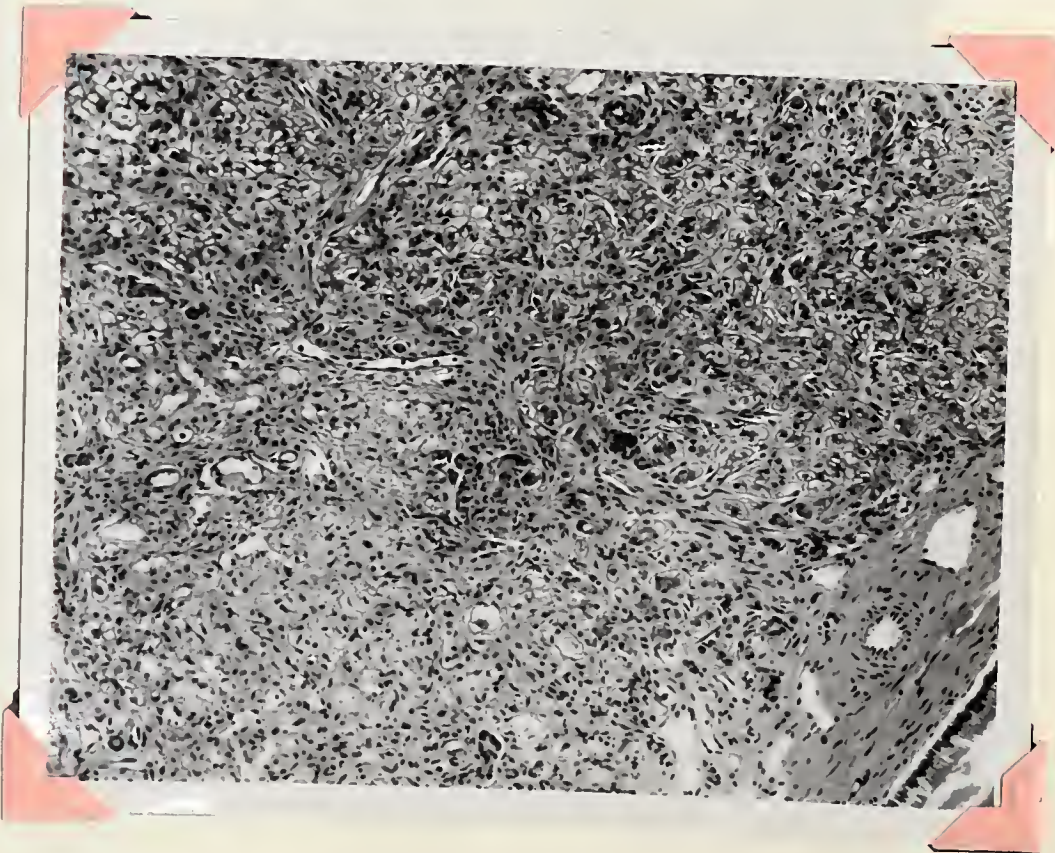


Fig. 24

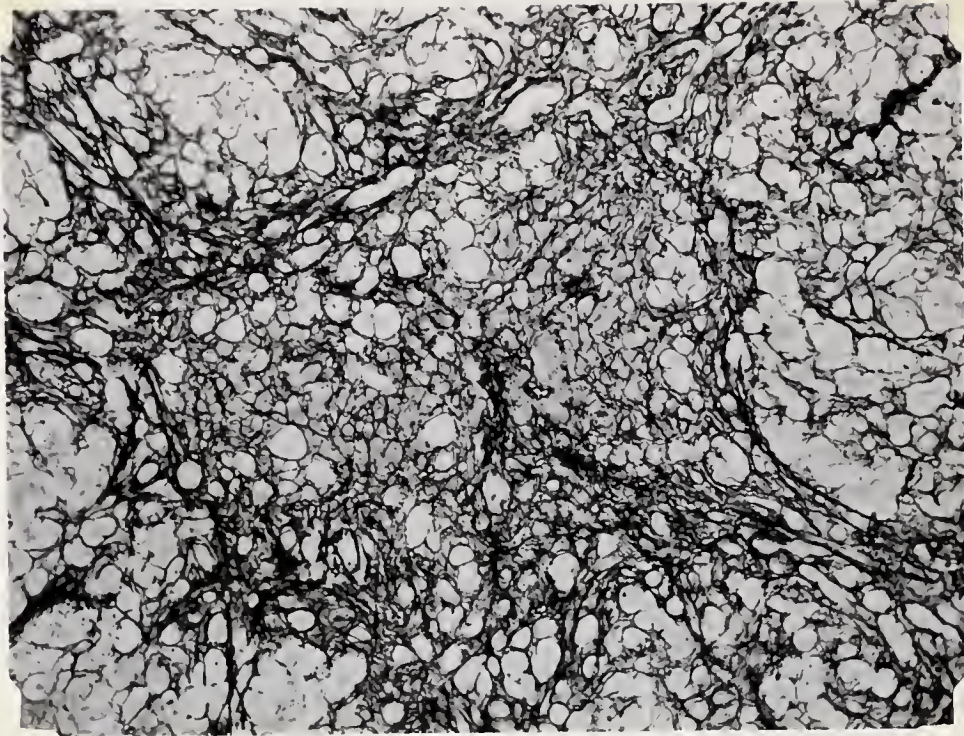


Fig. 25

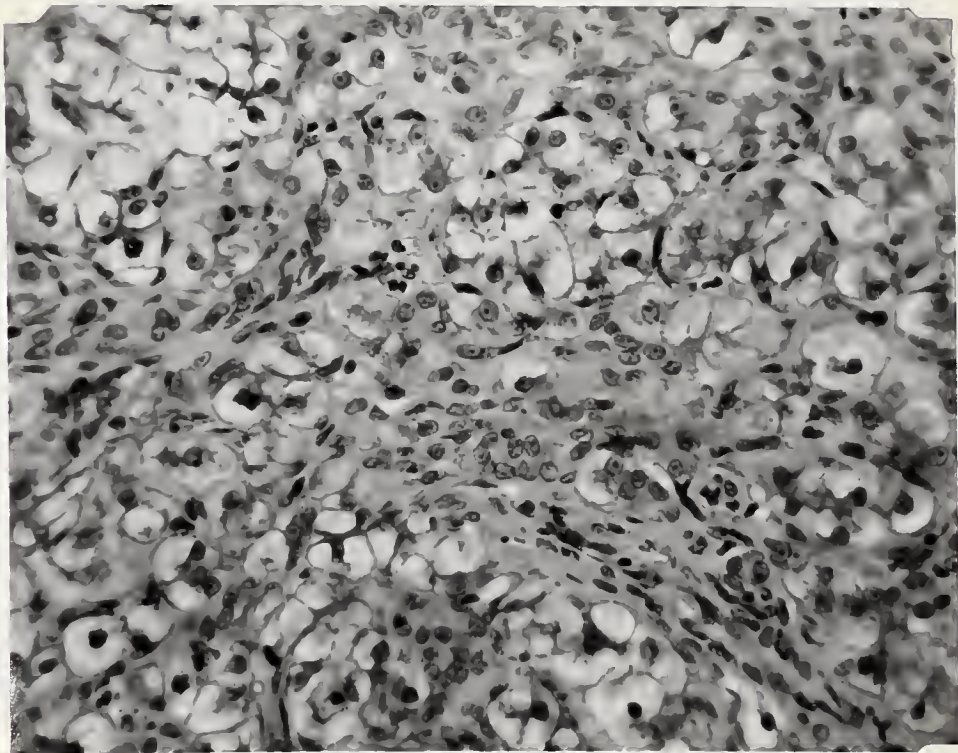


Fig. 26

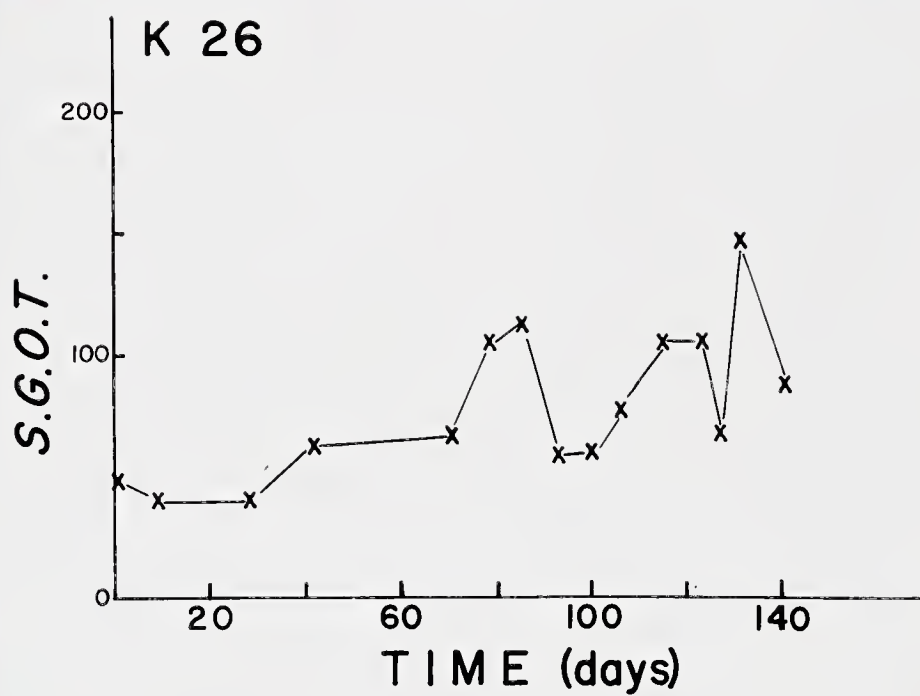


Fig. 27

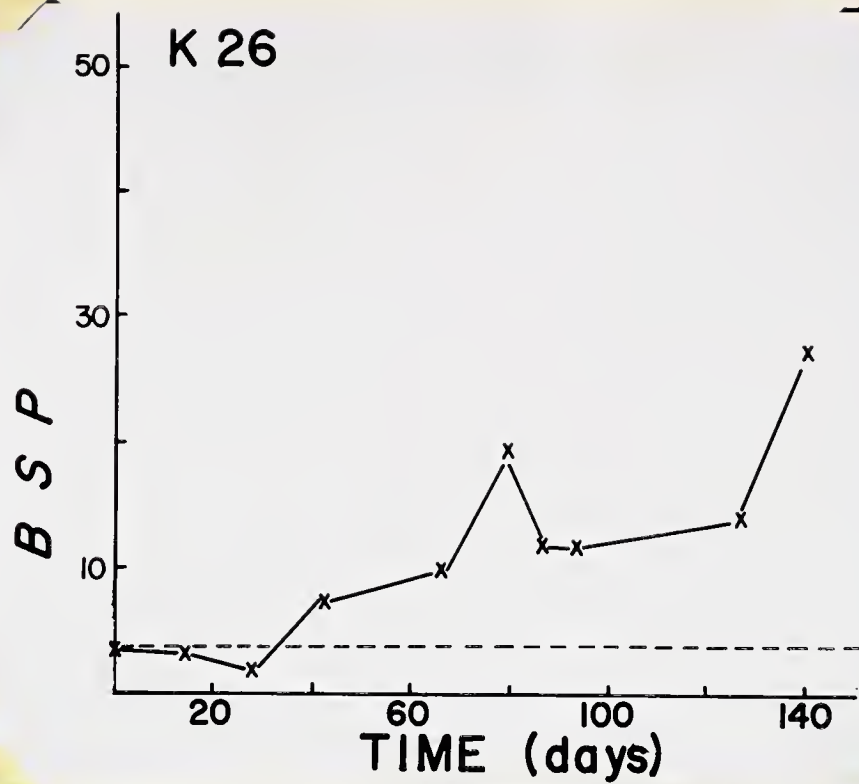


Fig. 28

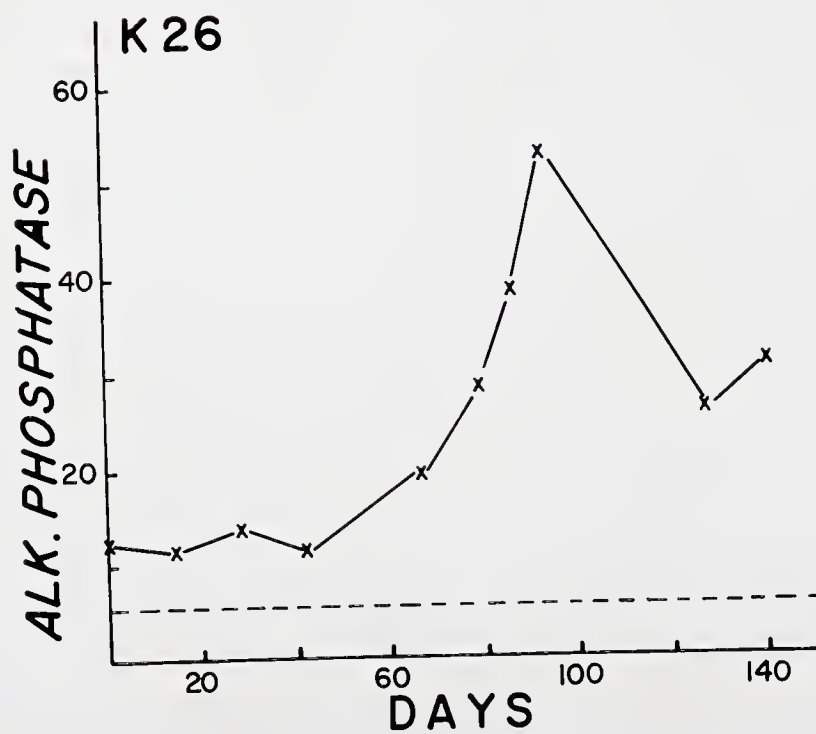


Fig. 29

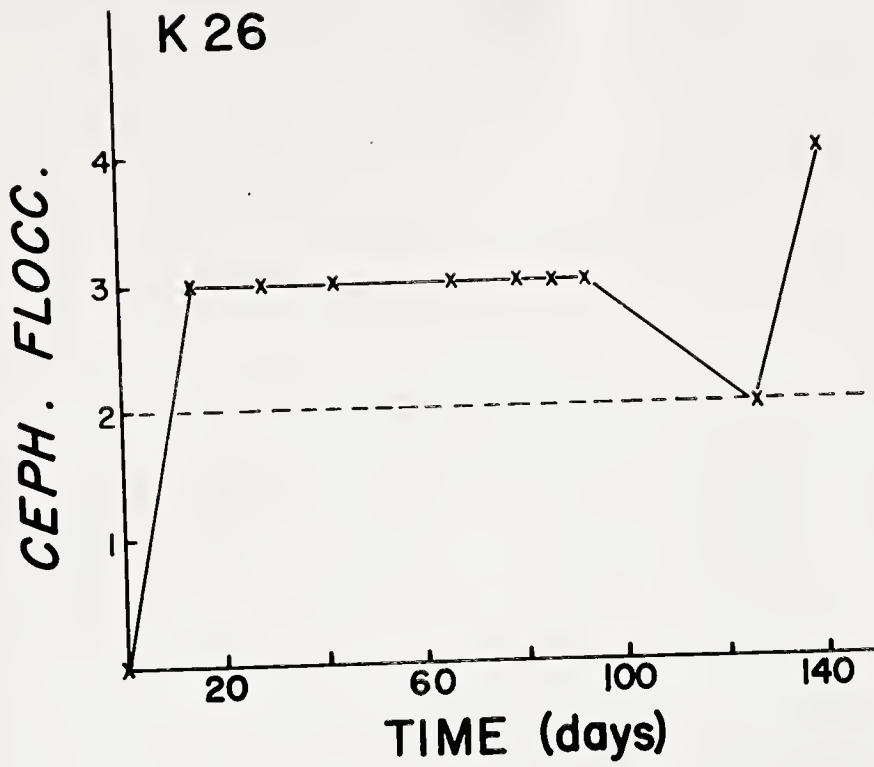


Fig. 30

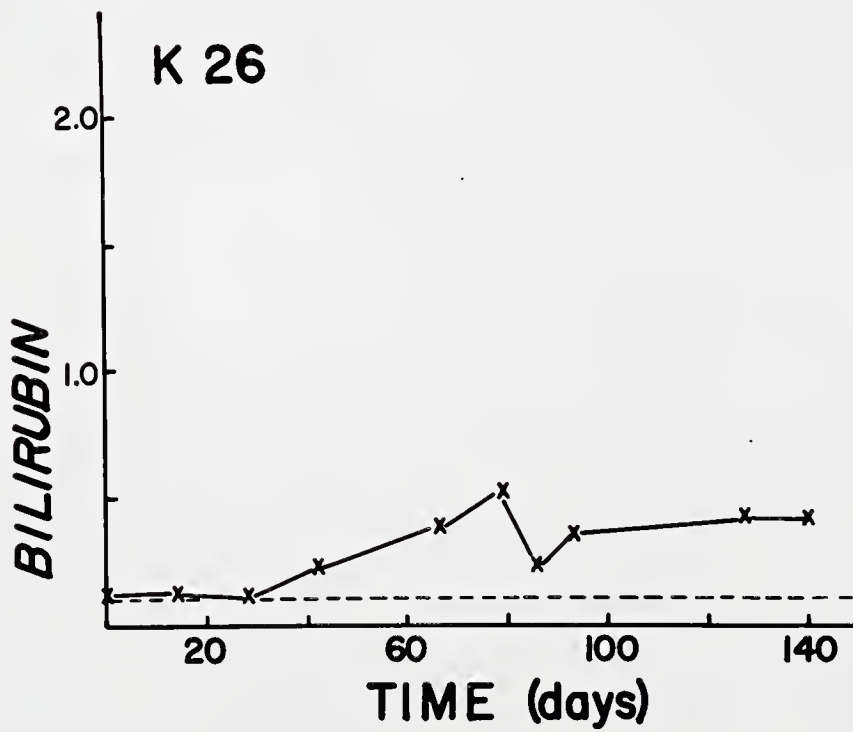


Fig. 31

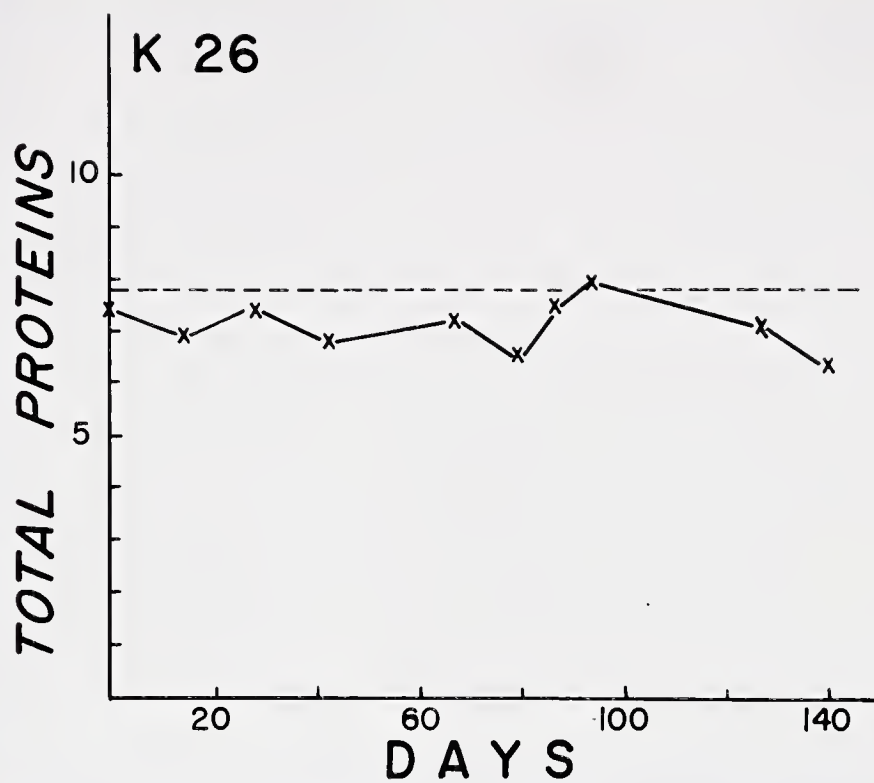


Fig. 32

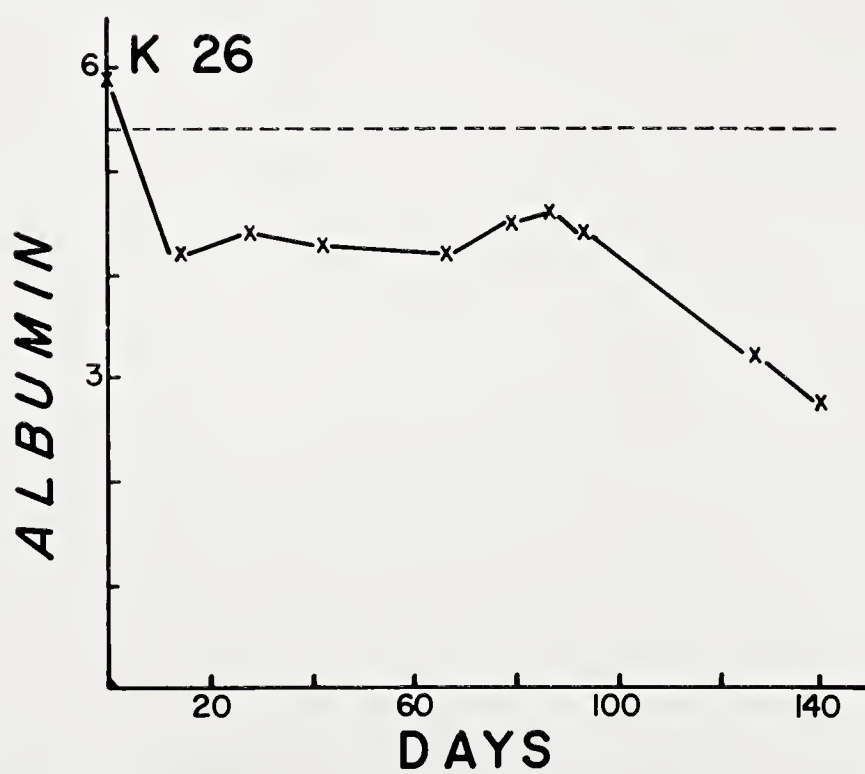


Fig. 33

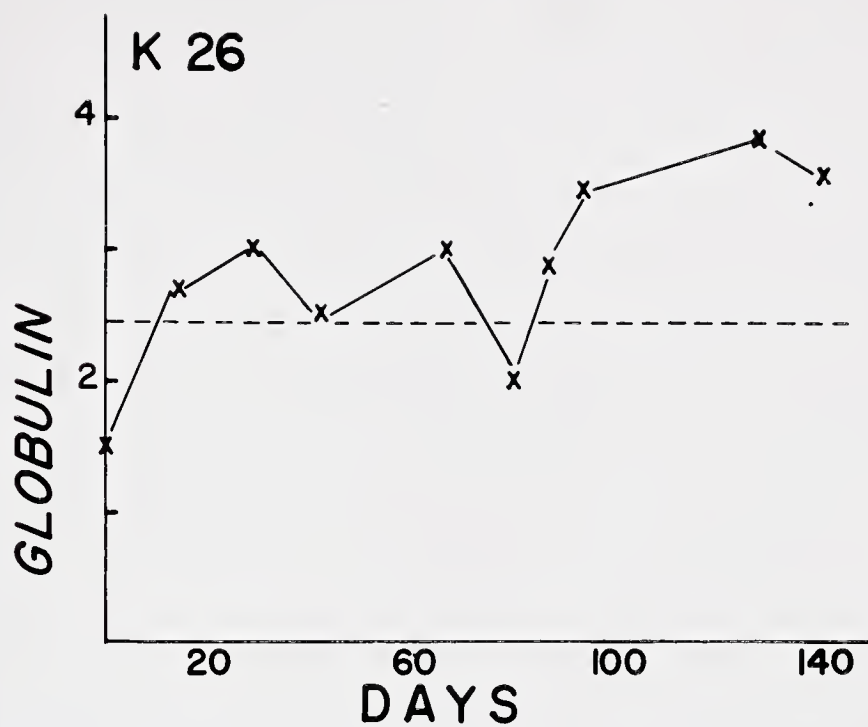


Fig. 34

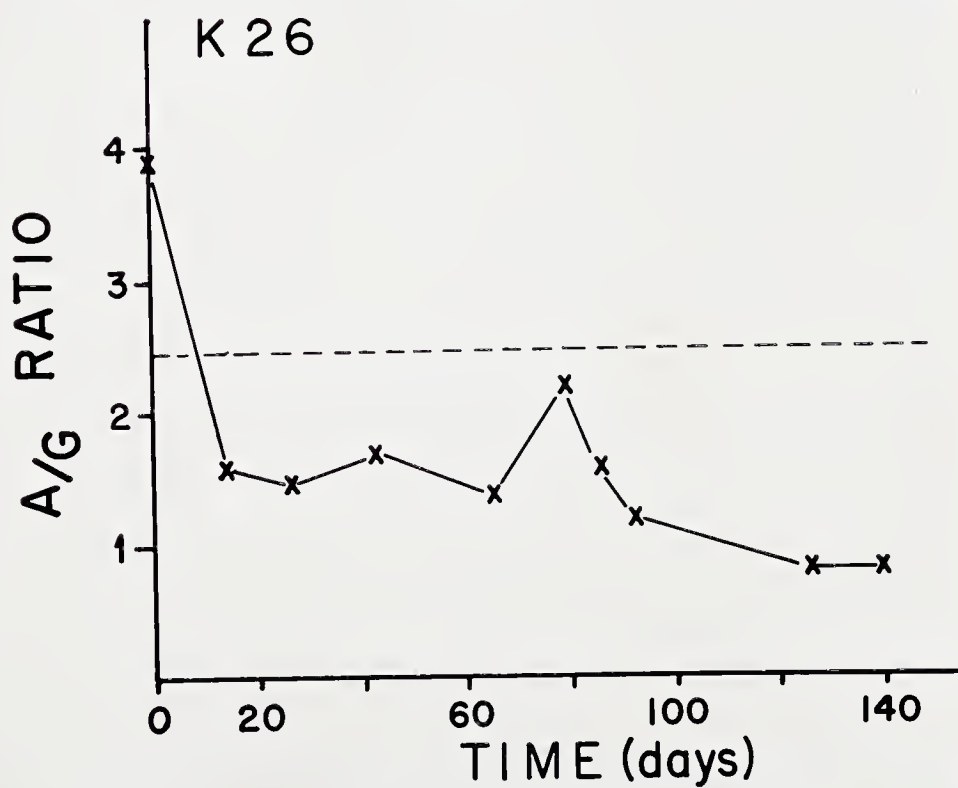


Fig. 35

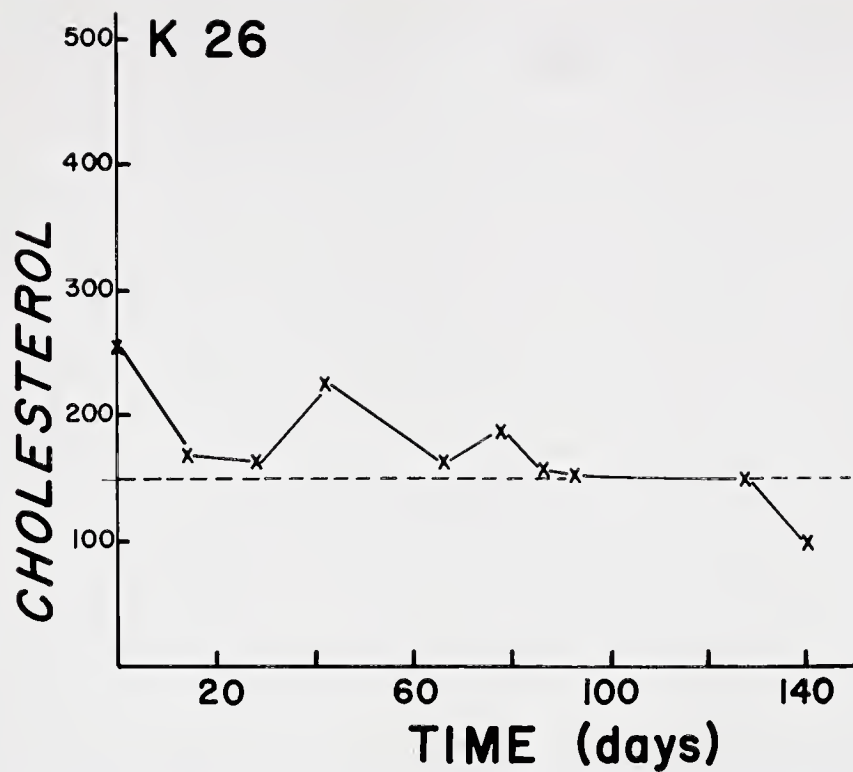


Fig. 36

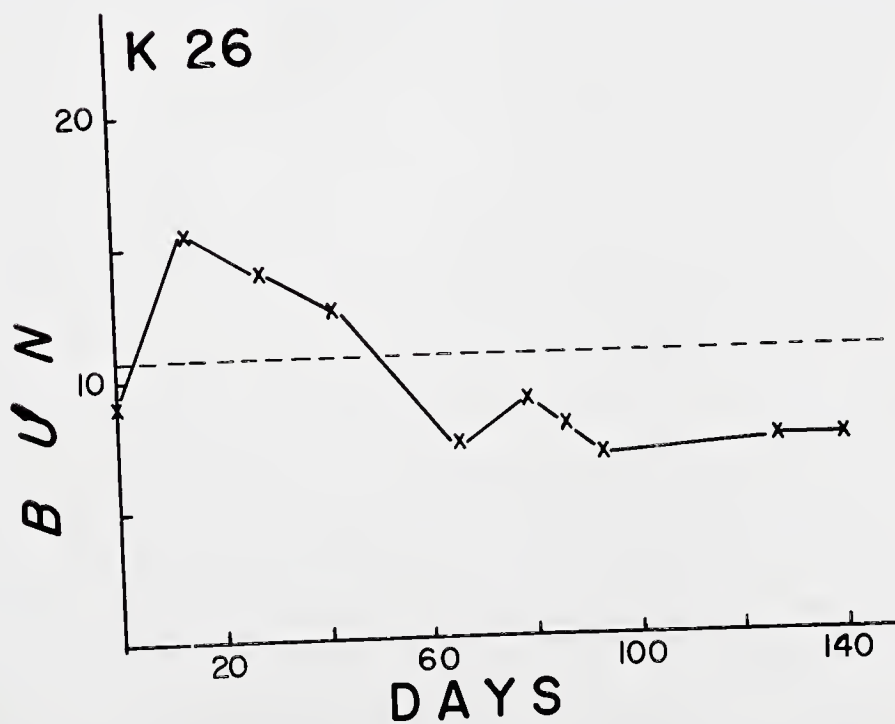


Fig. 37

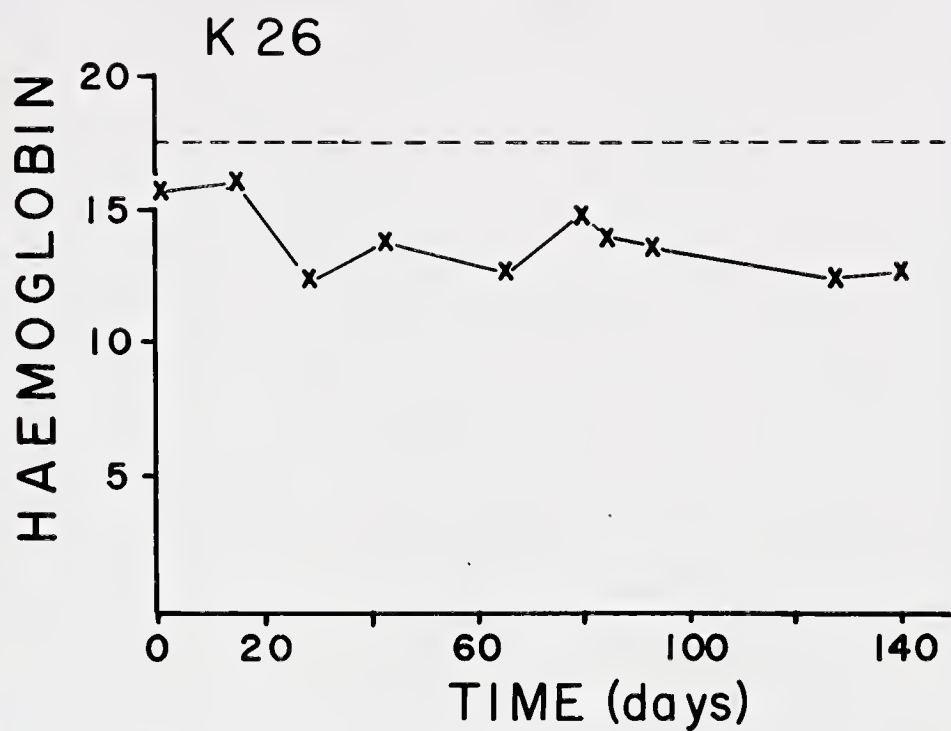


Fig. 38

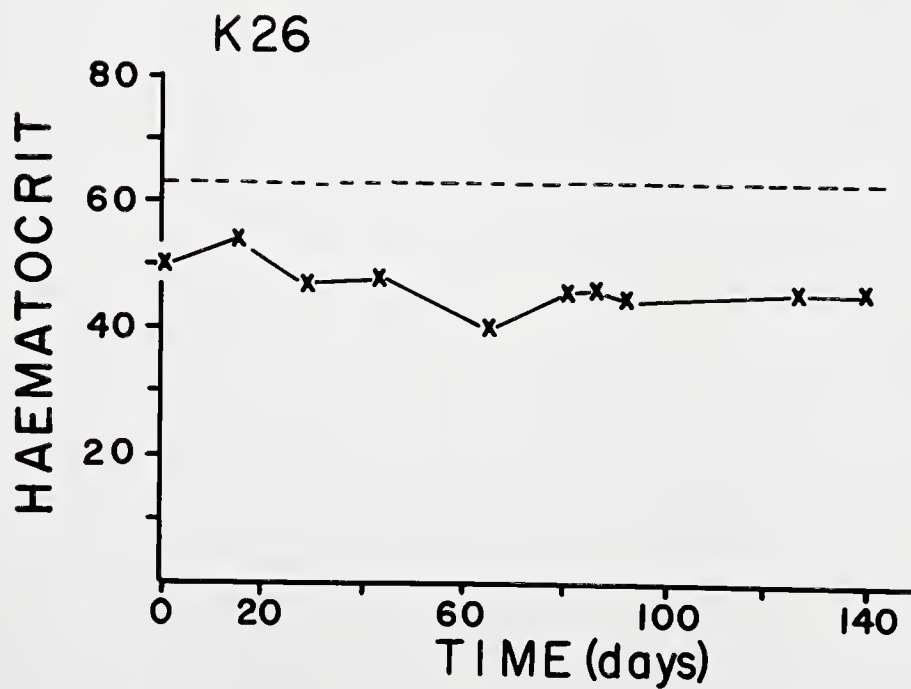


Fig. 39

DOG K-27

Hematological and biochemical investigation on the 16th of November, 1959, revealed no abnormality of liver function. Clinically the animal was alert, fit and healthy. Oral feeding with carbon tetrachloride in gelatin capsules commenced on the 18th of November, 1959. Initially the animal was given 1.5 ml. of carbon tetrachloride every second to third day. This was gradually increased until at the time of laparotomy 139 days after the start of the experiment, 10 ml. of carbon tetrachloride was being administered on alternate days. The dose of carbon tetrachloride was regulated by the level of SGOT. The aim was to keep the SGOT level below 100 units so that gross hepatic necrosis would be prevented. Over the period of 139 days a total of 251.25 ml. carbon tetrachloride was administered to the animal. During this period the dog remained well. During the first two months the animal's weight decreased by 1 kg. from an initial weight of 20.5 kg. Thereafter it gradually increased until at laparotomy it weighed 23 kg. Clinical examination revealed the presence of ascites some three weeks before laparotomy on the 5th of April, 1960,

139 days after the experiment began.

Anaesthetic, incision, procedure and closure were similar to that described for dog K-25.

Findings. Gross ascites was present, the fluid being of a clear straw colored character. The liver exhibited the gross appearances of advanced cirrhosis. It was small, nodular, deformed and light brown in color. The nodules which varied in size from 1 - 10 mm. in diameter were of a yellowish discoloration. The liver felt hard and this consistency was confirmed by incisional biopsy. The spleen appeared normal and there was no evidence of a portal systemic collateral circulation. No other abnormality was noted. The portal pressure measured 440 mm. saline.

HISTOPATHOLOGY

The hepatic architecture in this specimen is disorganized by a diffuse septal increase in connective tissue which has completely obscured the lobular pattern. The lobules have been broken up and disintegrated by the connective tissue encroachment, with the result that the numerous smaller areas of hepatic cells, pseudo-lobules in fact, are present diffusely throughout the tissue. In some areas a centrolobular necrotic process is discernible. Many of the nodules and pseudo-lobules themselves are the seat of septal fibrosis.

The increase in connective tissue is probably both "active" and "passive", but its diffuse septal character and the appearance of fibroblasts and collagen suggest that most of it is new and "active".

In detail, the fibrosis is found to be (1) Centrolobular, (2) Intraparenchymal, (3) Perilobular, (4) Portal, (5) Periportal and (6) Periductular for there is an increase in the amount of fibrous tissue surrounding the numerous actively regenerating bile ductules. These bile ductules occur mainly in the portal and periportal tracts, but are found whenever there is evidence of an increase in connective tissue formation. These regions are also the seat of an inflammatory cell infiltration, mainly of the monocytic variety.

The nodules of hepatic cells, surrounded by fibrous tissue septums are actively regenerating, showing evidence of thickened cellular layers, mitosis and increased cell size. Some of the nodules are moderately large, but never exceeding a few millimeters in diameter.

The reticulum stain demonstrates quite clearly the gross and diffuse disorganization of the lobular architecture, showing also the regenerating nodules with their "passive" septums at the periphery, and the exceedingly irregular fragmentation of the liver structure.

There seems to be very little doubt that this specimen demonstrates the classical picture of portal or septal cirrhosis, in which there is disorganization of structure, nodular regeneration and a diffuse septal increase in connective tissue.

In this animal, the advanced degree of cirrhosis has resulted in gross ascites and portal hypertension of marked degree. (See Figs. 40-43).

BIOCHEMISTRY

Serum Alkaline Phosphatase, Serum Bilirubin, Serum Cephalin flocculation and BSP retention bore a directly proportional relationship to the level of SGOT during the experiment. All the above mentioned parameters gradually rose to levels which were significantly abnormal. The Total Protein level fluctuated slightly within the limits of normality, while the Serum Globulin demonstrated a final increase and the Serum Albumin a final decrease. The Albumin Globulin ration was finally reversed.

Serum Cholesterol demonstrated a gradual and constant decrease as the experiment proceeded.

The BUN fluctuated within the limits of normality, the general tendency being towards a gradual decrease. (See Figs. 44-54).

HEMATOLOGY

Both Hemoglobin and Hematocrit estimations showed a gradual but slight decrease which remained within the limits of normality. See Figs. 55, 56).



Fig. 40

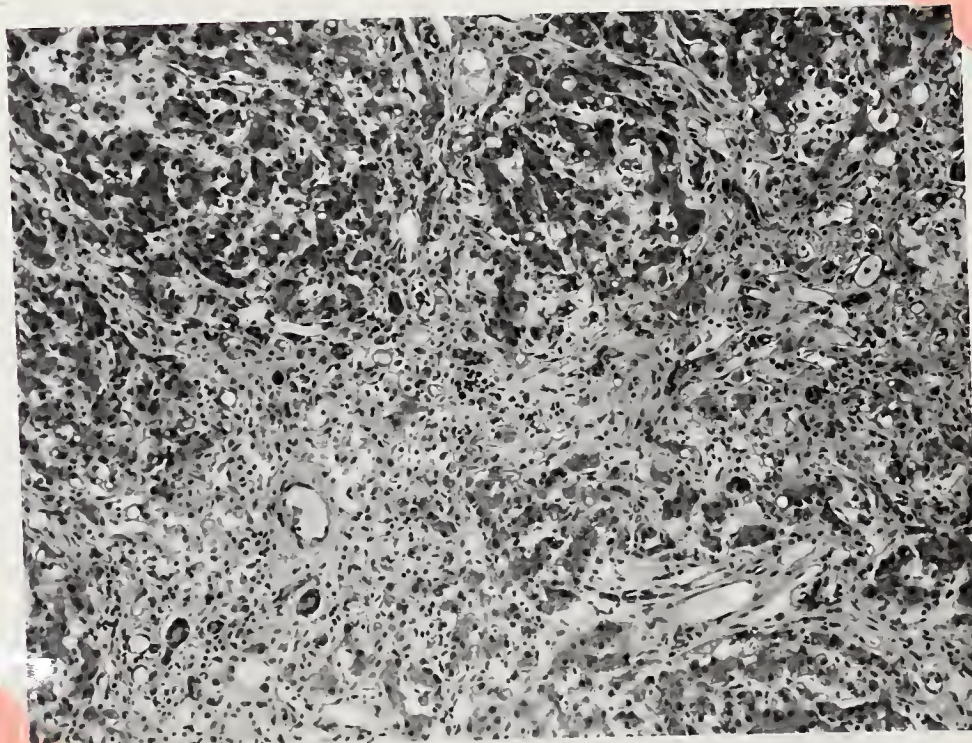


Fig. 41

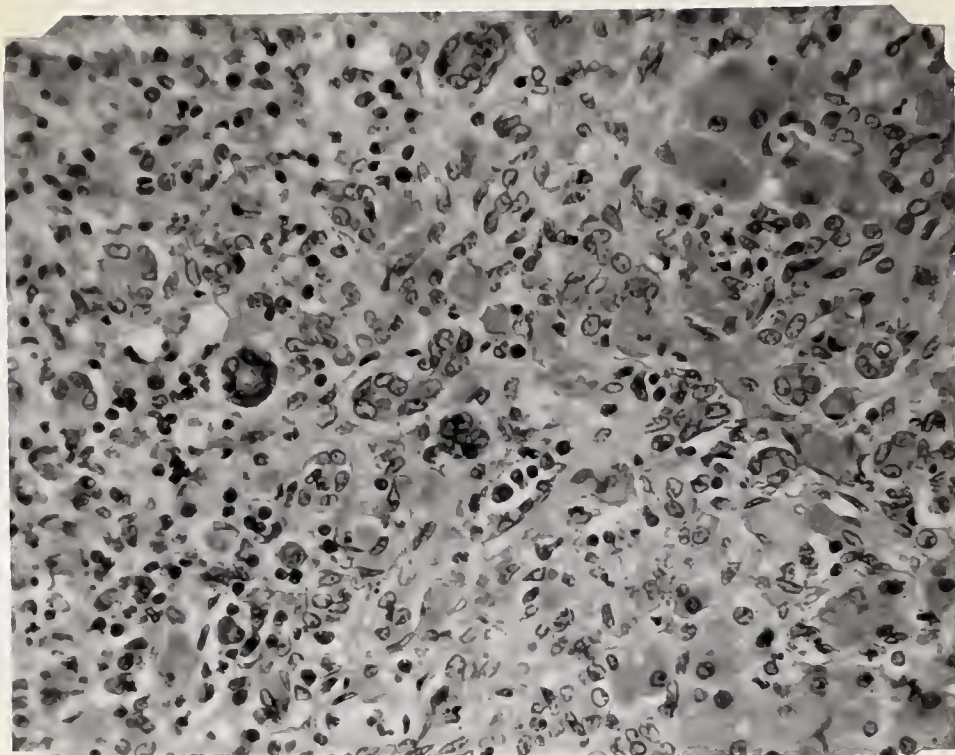


Fig. 42

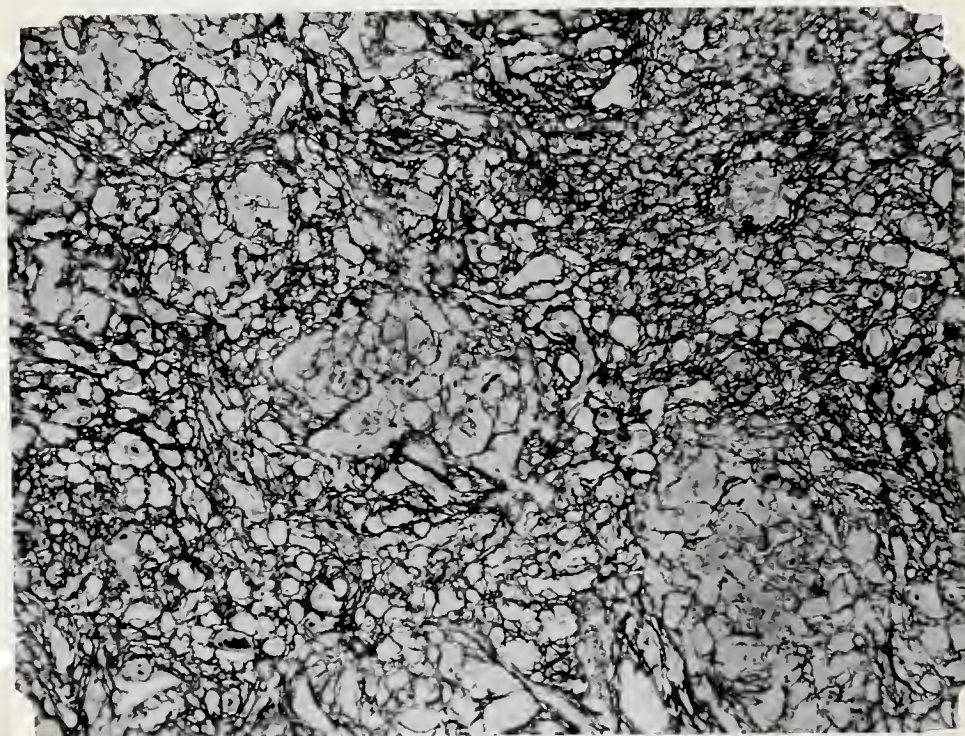


Fig. 43

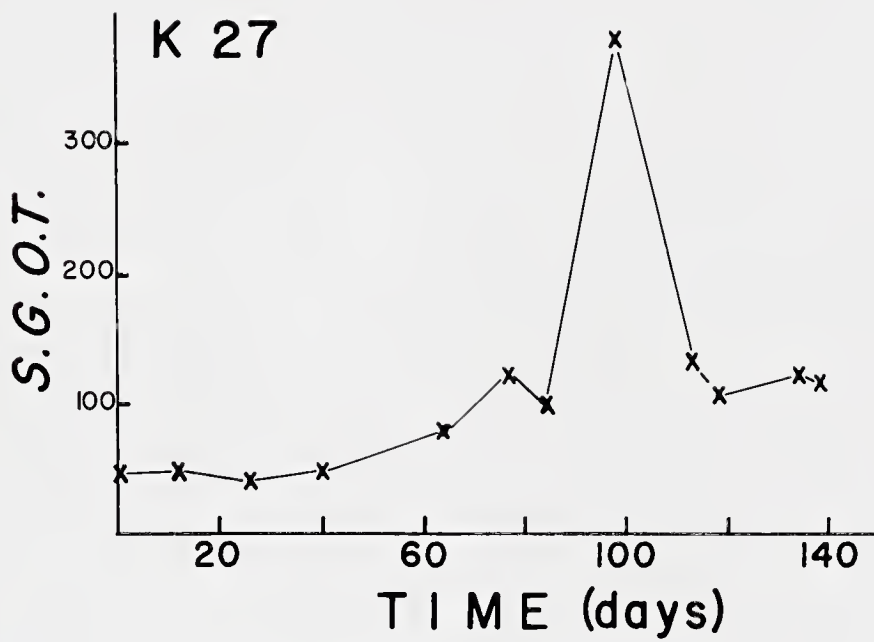


Fig. 44

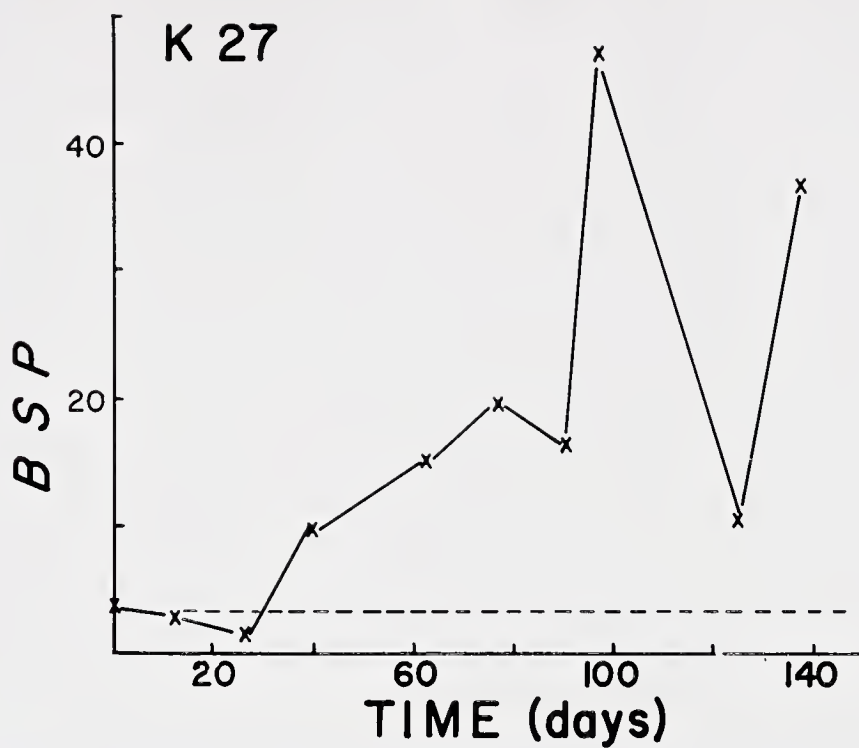


Fig. 45

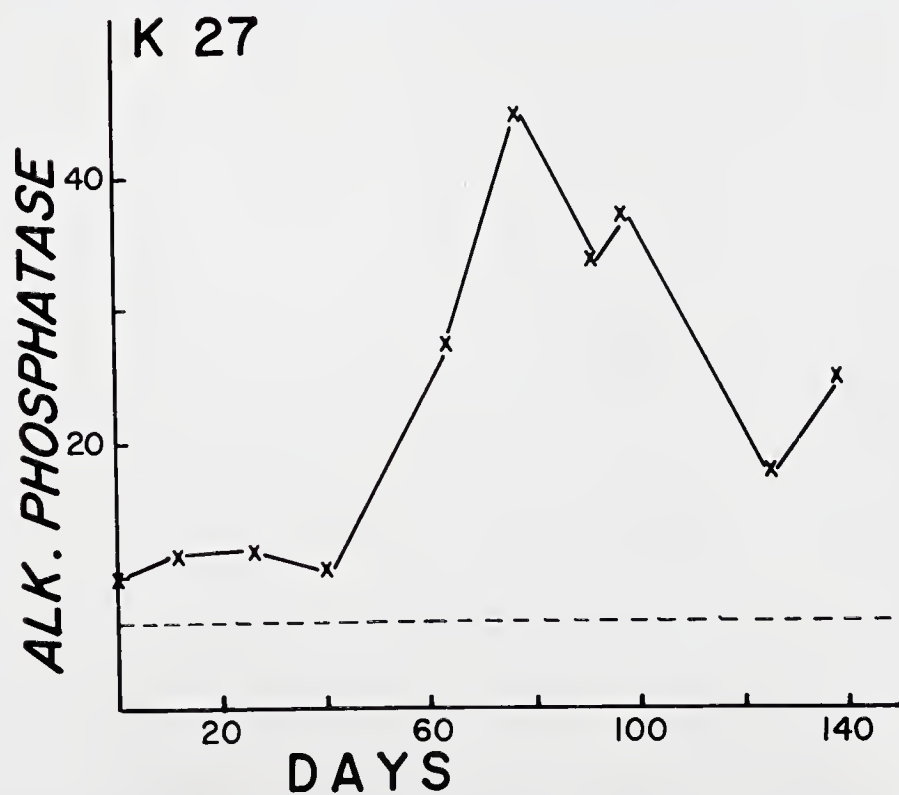


Fig. 46

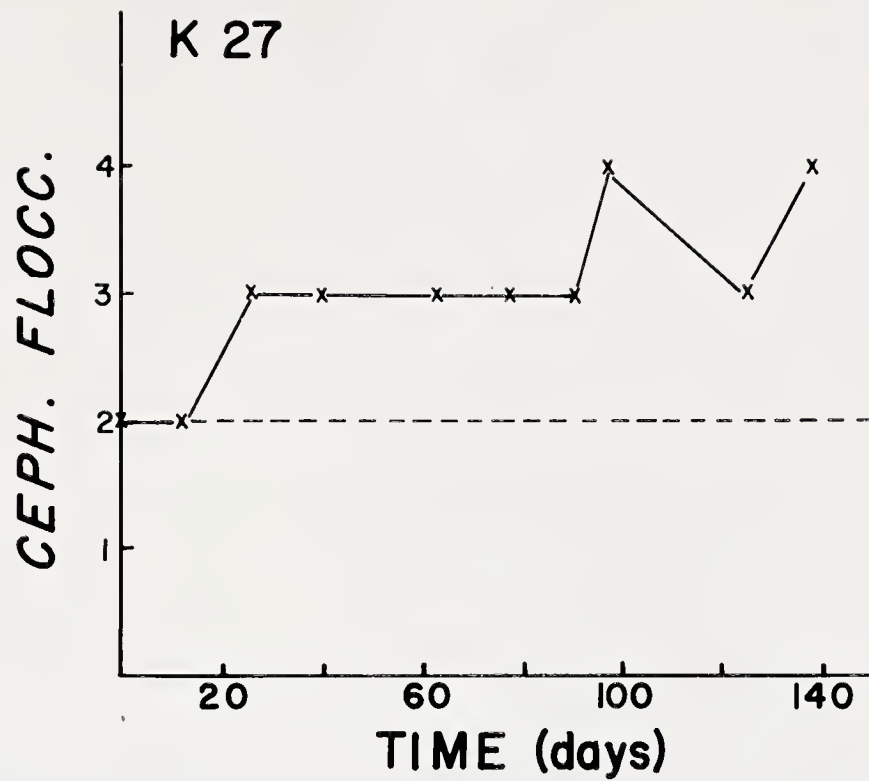


Fig. 47

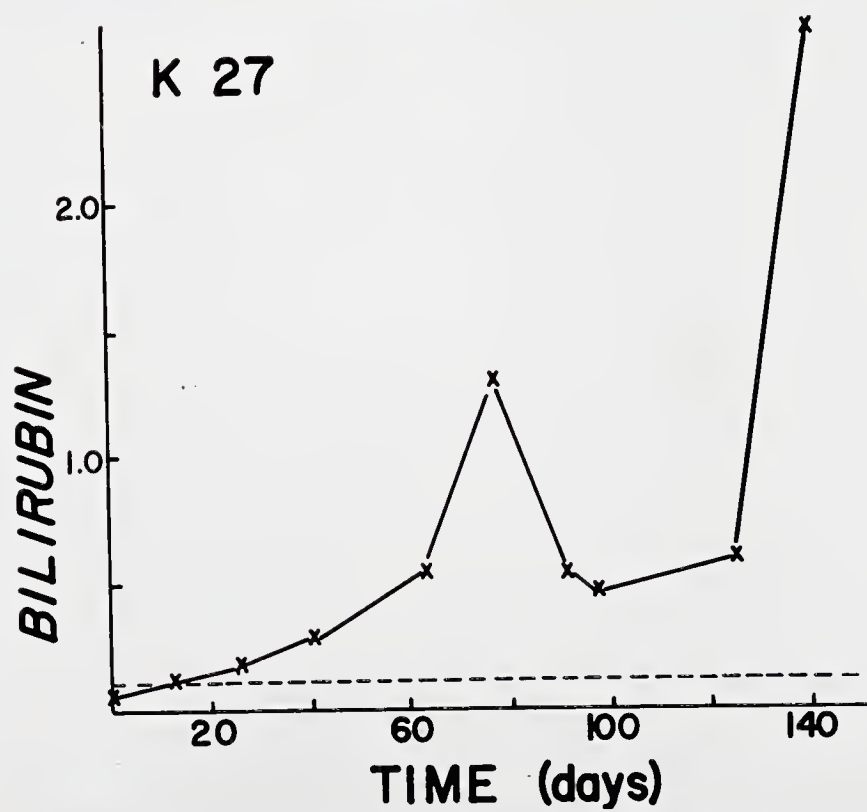


Fig. 48

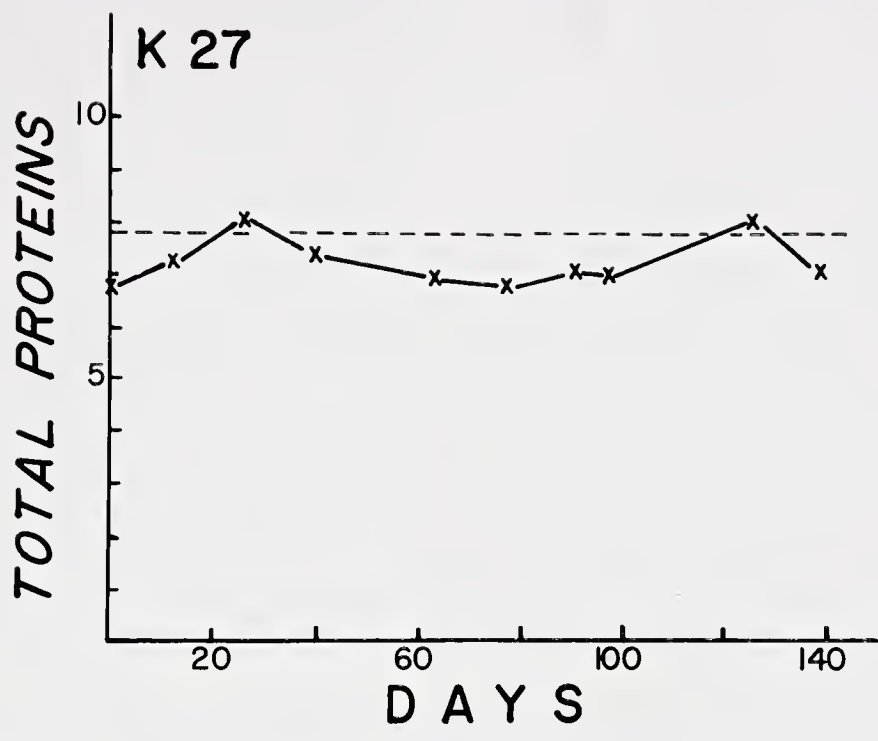


Fig. 49

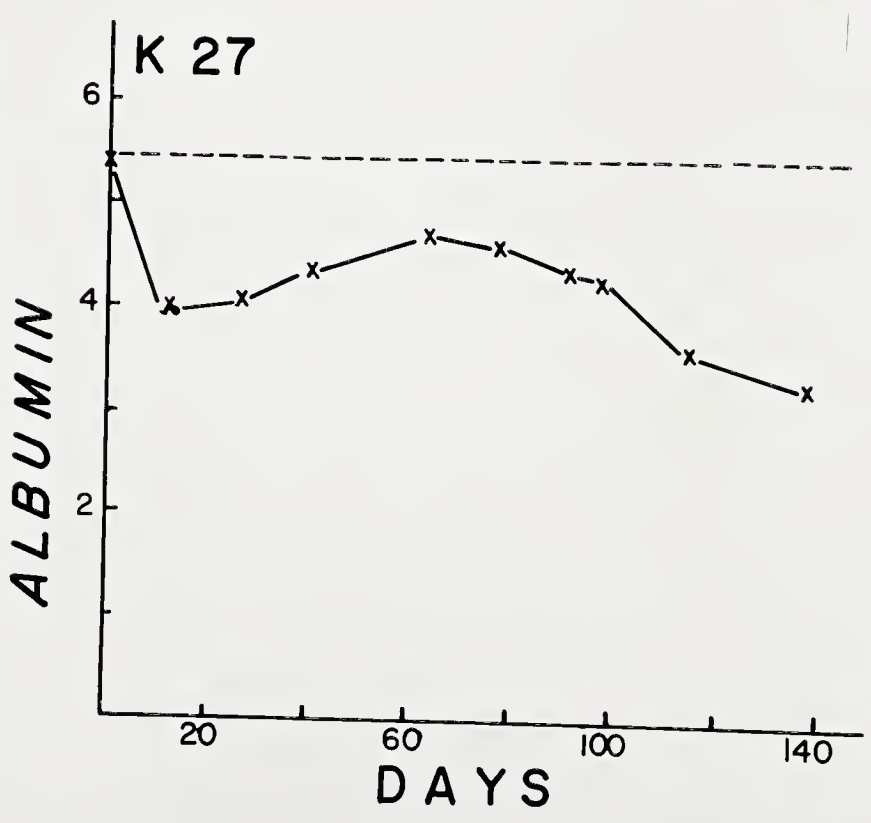


Fig. 50

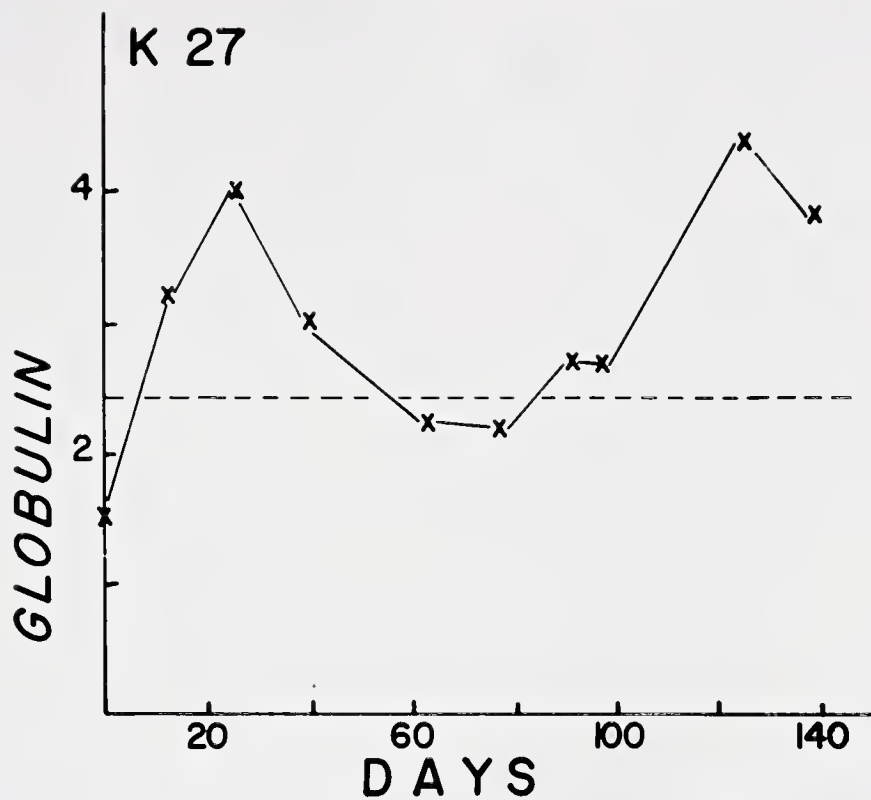


Fig. 51

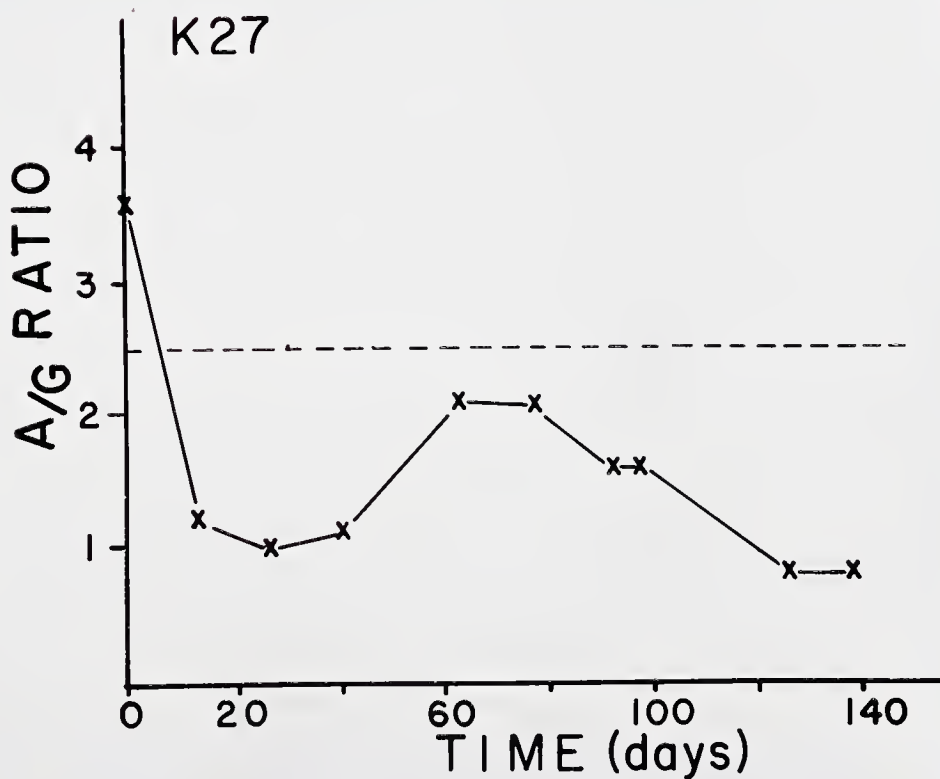


Fig. 52

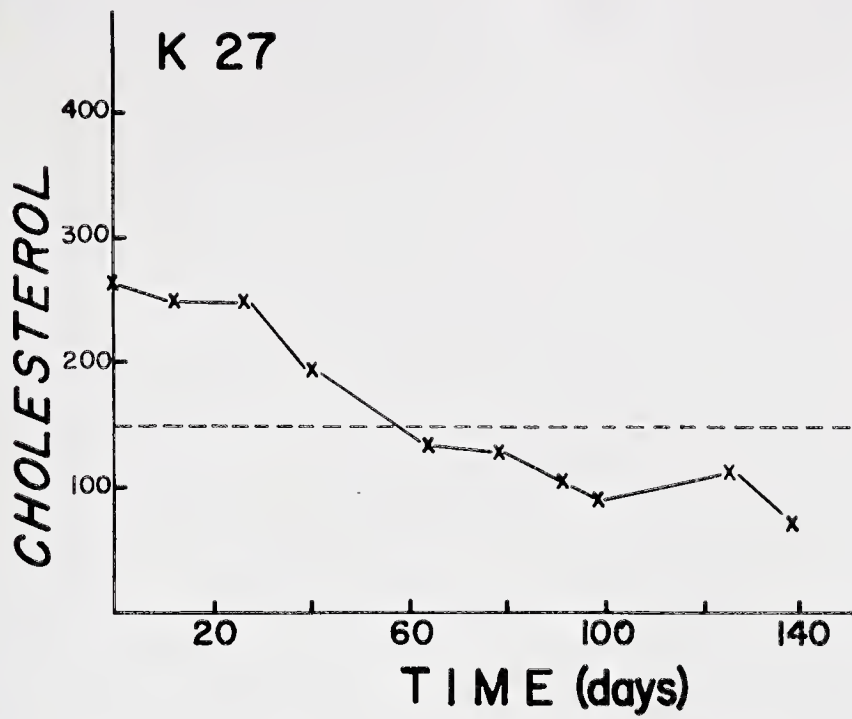


Fig. 53

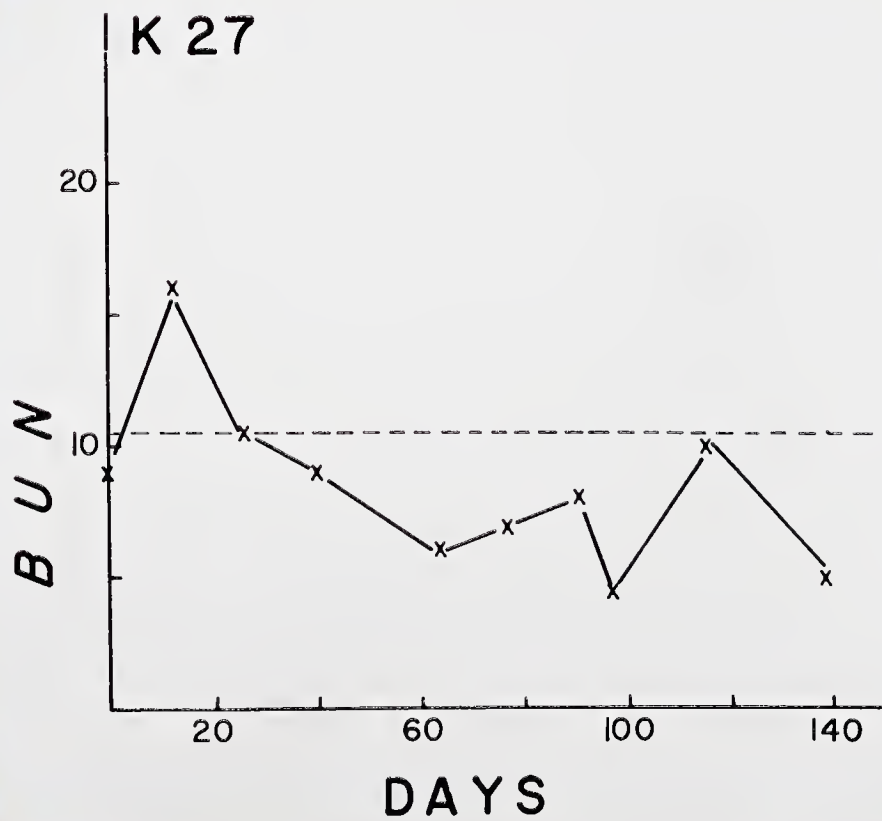


Fig. 54

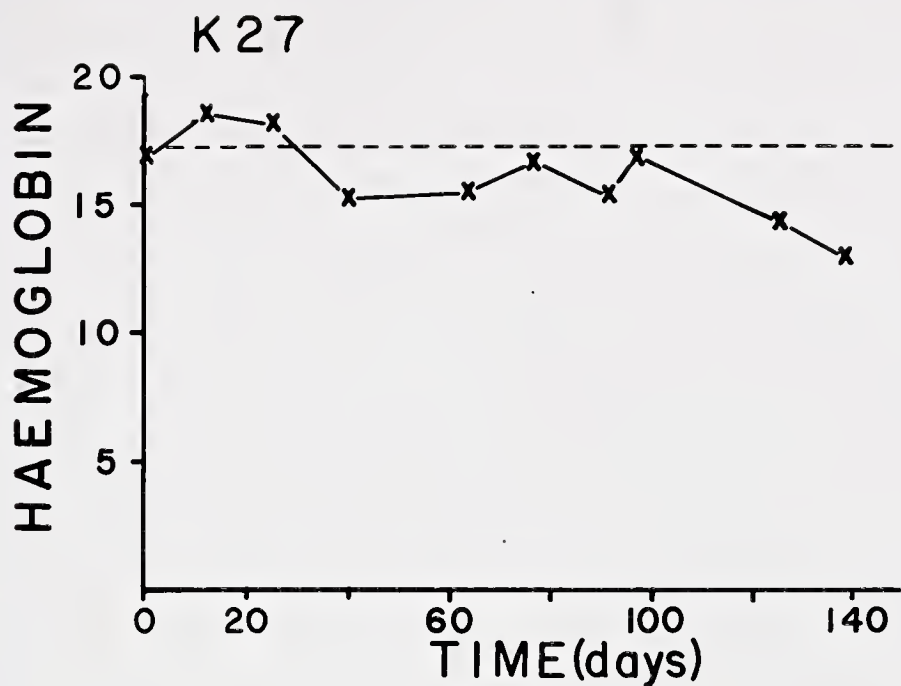


Fig. 55

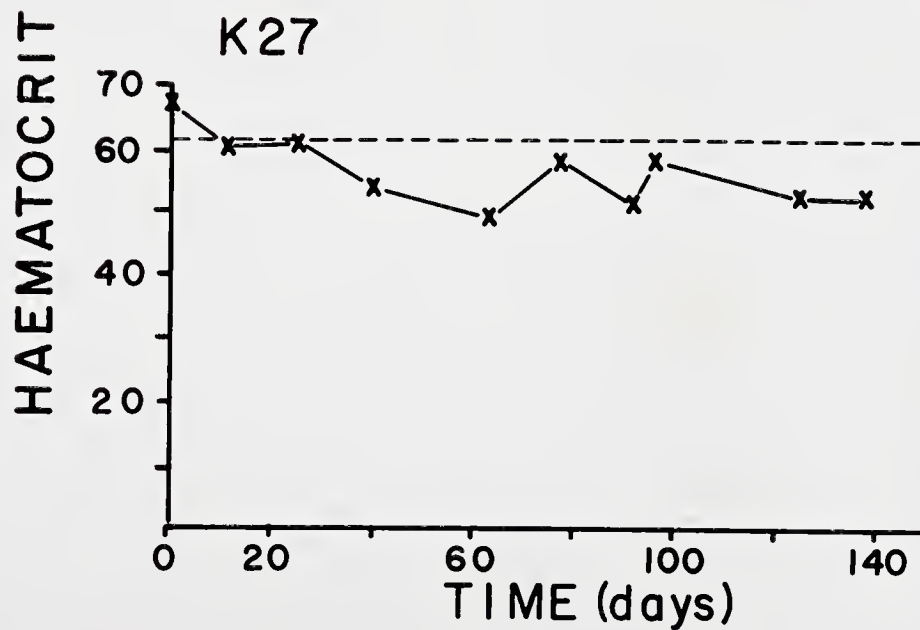


Fig. 56

DOG K-28

Hematological and biochemical investigation on the 20th of November, 1959, revealed no abnormality of liver function. Clinically the animal was alert, fit and healthy. Oral feeding with carbon tetrachloride in gelatin capsules commenced on the 20th of November, 1959, after the blood for investigations had been taken. Initially the animal was given 1.5 ml. carbon tetrachloride every second to third day. This was gradually increased until at the time of laparotomy 138 days after the start of the experiment, 10 ml. carbon tetrachloride was being administered on alternate days. The dose of carbon tetrachloride was regulated by the level of SGOT. The aim was to keep the SGOT level below 100 units so that gross necrosis would be prevented. Over the period of 138 days a total of 242 ml. carbon tetrachloride was administered. During this period the dog remained healthy and increased his weight from 13.5 kg. initially to 17.5 kg. at the time of laparotomy, which was performed on the 6th of April, 1960, 138 days after the experiment began. The surgical procedure was performed under aseptic conditions and the anaesthetic, incision, procedure and closure were similar to that described for dog K-25.

FINDINGS

The liver appeared smaller than would be expected for a dog of this weight. It was generally pale in color, rubbery in consistence and with a finely, granular, nodular surface. The little granular areas were paler in color than the rest of the liver surface. The spleen was normal in appearance and size. There was no evidence of a portal systemic collateral circulation, nor was ascites present. No other abnormality was noted. The portal pressure measured 260 mm. saline.

HISTOPATHOLOGY

This specimen presents numerous abnormalities, the most obvious and widespread being a diffuse centrolobular degeneration and necrosis. In some areas the necrosis is more vigorous involving almost entire lobules, leaving only a small amount of periportal hepatocellular tissue.

Throughout the specimens examined there are sheets of connective tissue in relation to the portal and centrolobular areas. In some areas, particularly the centrolobular portions, the appearance of increase in fibrosis is most likely due to collapse and compression of necrossed cells, but in others the increase seems active and new for the sheets stain deeply and are filled with healthy looking fibroblasts. In these last mentioned areas, cellular necrosis is not so evident and

the lobular architecture is broken up by these connective tissue septums. However, the disorganization of the lobular pattern is never gross.

In the regions where centrolobular necrosis is severe, the midlobular fields usually show fairly marked vacuolation, probably indicative of fatty degeneration or infiltration.

With sparing of the periportal and peripheral lobular zones, some regions are suggestive of hypertrophic nodule formation, with portal tracts at their centers. This appearance however is not frequently seen.

In some periportal areas, evidence of beginning regeneration is noted in the form of cell layers two or more cells thick with an increase in mitosis and the presence of binucleate cells. A moderate increase in bile duct proliferation is noted in the portal tracts.

The specimen stained for reticulum demonstrates an increase in reticulum in the centrolobular, portal and periportal regions, as well as an irregular intraparenchymal increase. Broad bands of reticulum staining material connect the centrolobular and portal areas and there is frequently an increase in bands connecting centrolobular areas which produce a "passive" septum effect around periportal areas.

There is a faint suggestion of early lobular

disintegration. Little inflammatory cell infiltration is noted.

This specimen demonstrates mainly an hepatocellular degeneration and necrosis of the centrolobular variety. There is also early evidence of a cirrhotic type of reaction by the liver, which though slight, is of the diffuse variety.

Although this animal received one of the largest quantities of carbon tetrachloride over the longest period, the effects on the liver may be described as slight when compared with the other experimental animals. This appears to be another example of resistance to the toxin by the body of the dog generally and the hepatic cells in particular. (See Figs. 57-60).

BIOCHEMISTRY

BSP retention was noted to relate in a directly proportional manner to the level of SGOT. During the latter half of the experiment it was difficult to maintain the SGOT below 100 units. However, the dog remained well throughout this period.

The Total Serum Protein level varied little, while Serum Albumin decreased slightly, Serum Globulin increased slightly, both nonsignificantly. The Albumin-Globulin ratio fluctuated somewhat, the final result being a small reduction. Cephalin flocculation

increased gradually and significantly.

The Serum Cholesterol level rose slightly, while BSP retention and Serum Alkaline Phosphatase levels rose markedly. The Serum Bilirubin level demonstrated a gradual increase through the period of the project, but never reaching an abnormal level.

The BUN level fluctuated moderately, finally decreasing slightly but not significantly. (See Figs. 61 - 71)

HEMATOLOGY

No significant change occurred with Hemoglobin and Hematocrit estimations during the period of the experiment. (See Figs. 72, 73).



Fig. 57

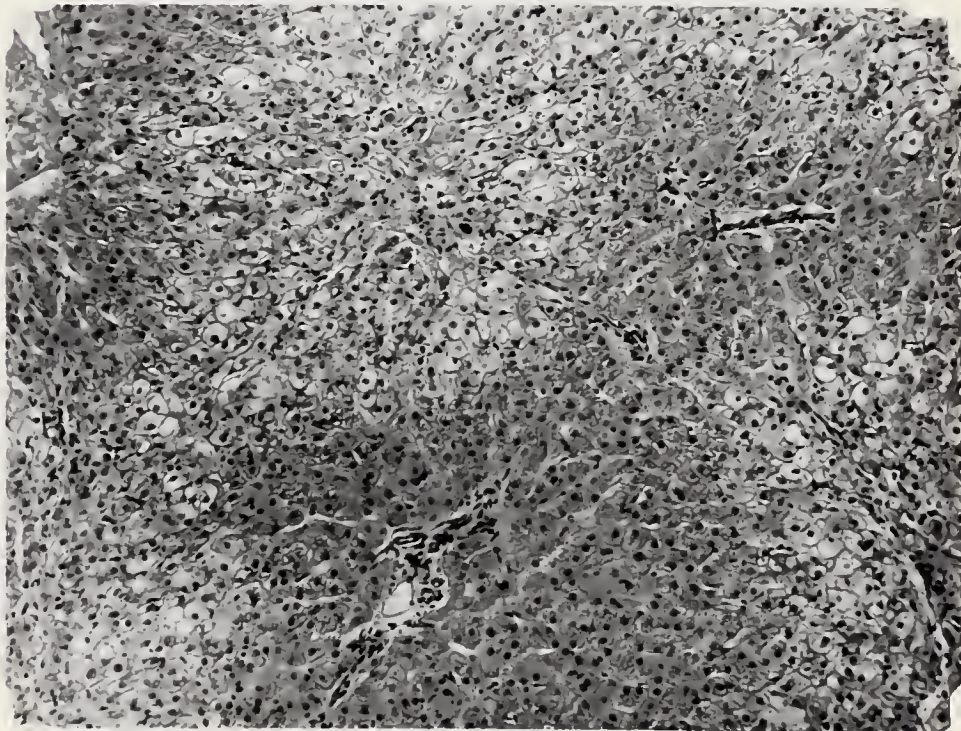


Fig. 58

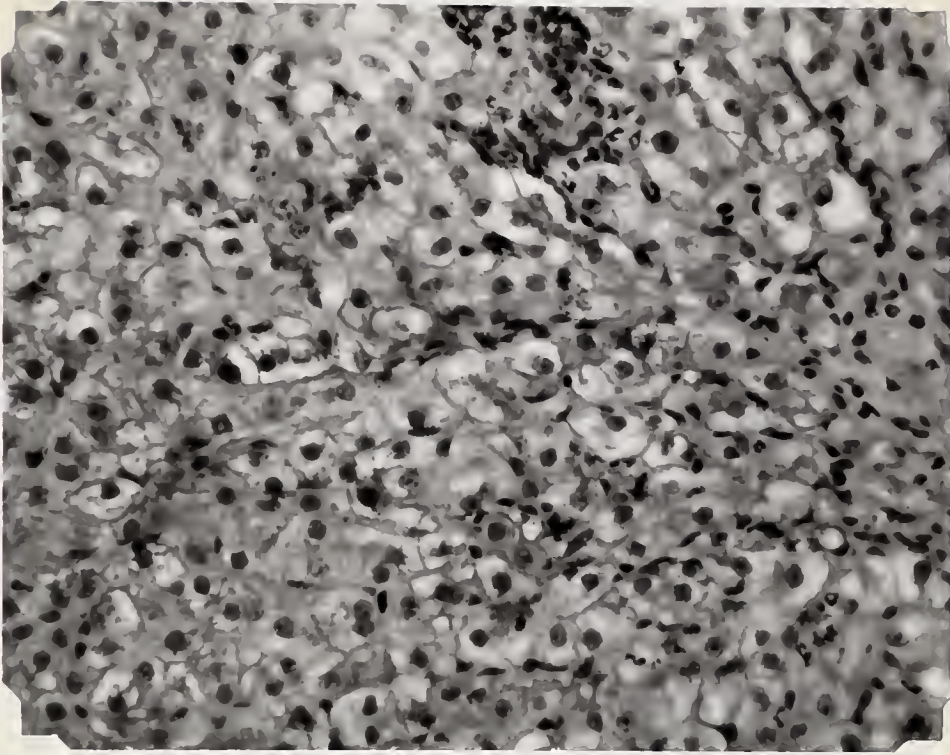


Fig. 59

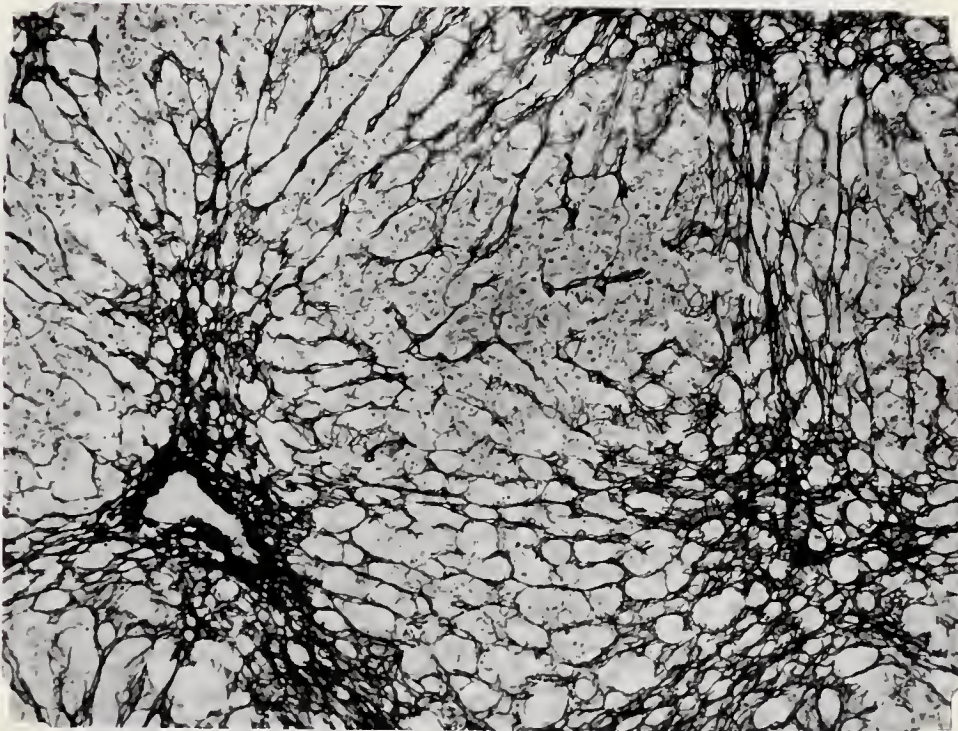


Fig. 60

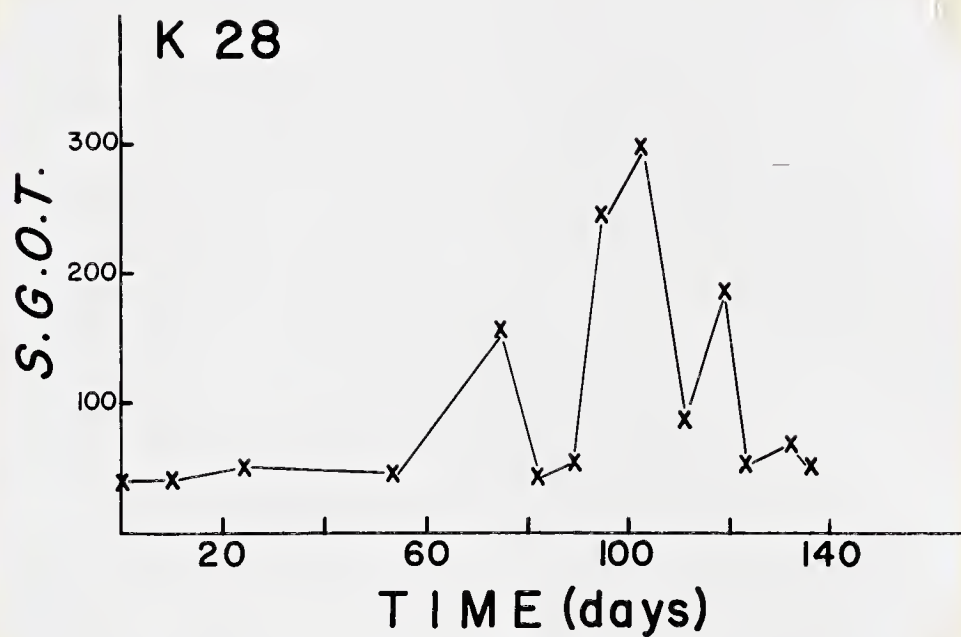


Fig. 61

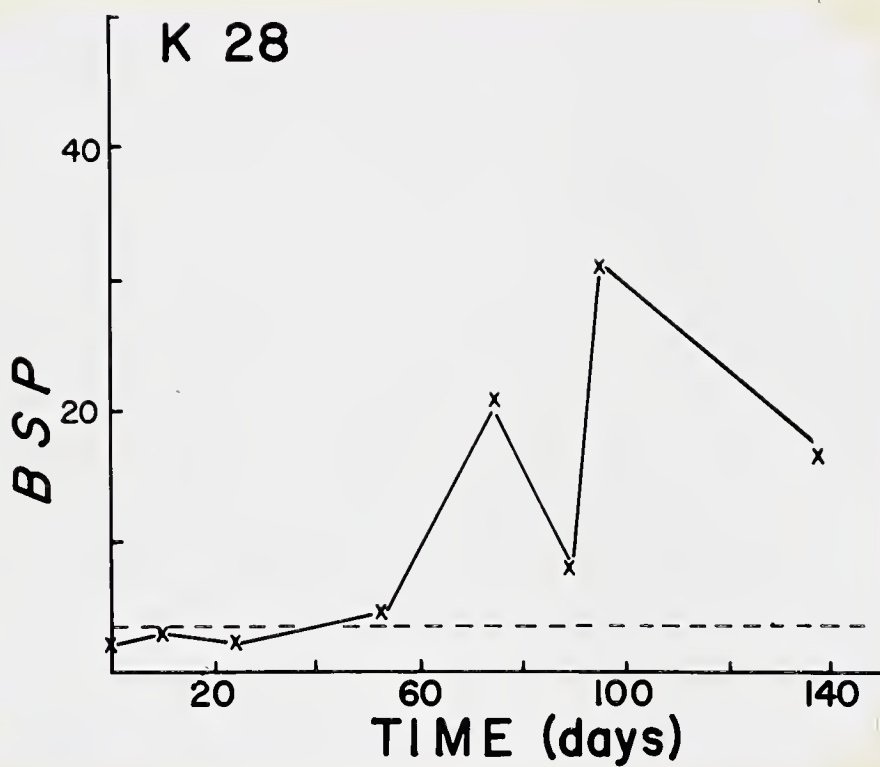


Fig. 62

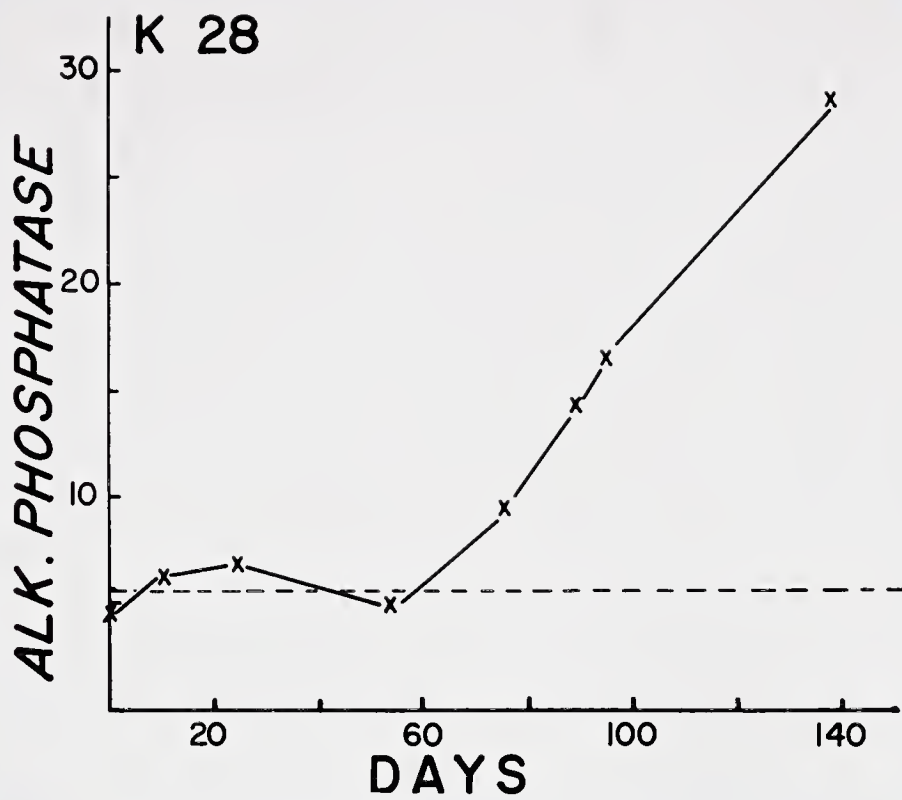


Fig. 63

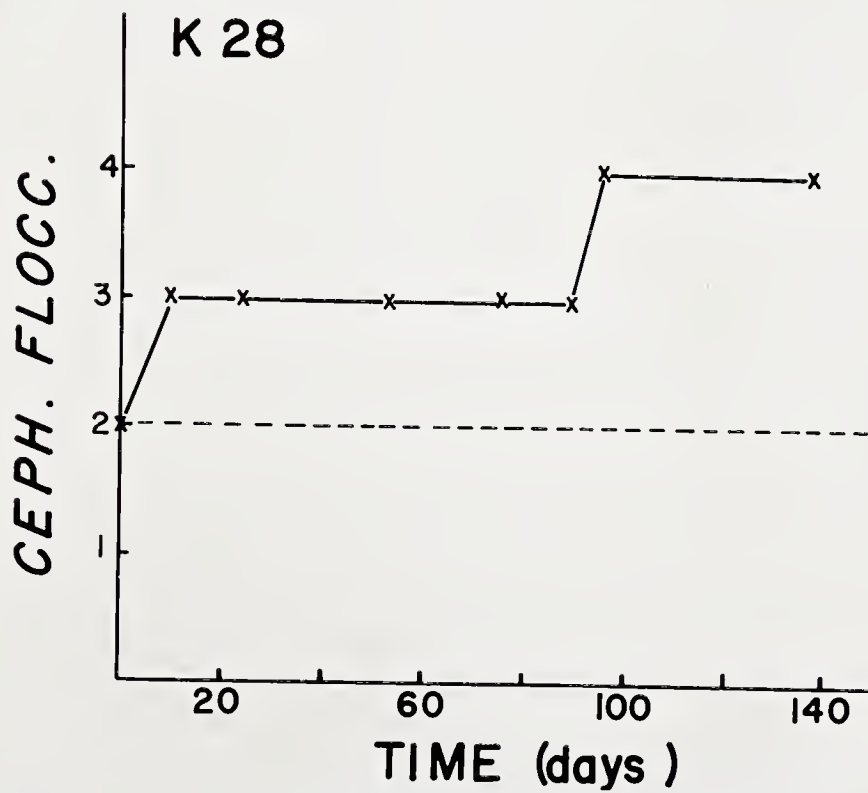


Fig. 64

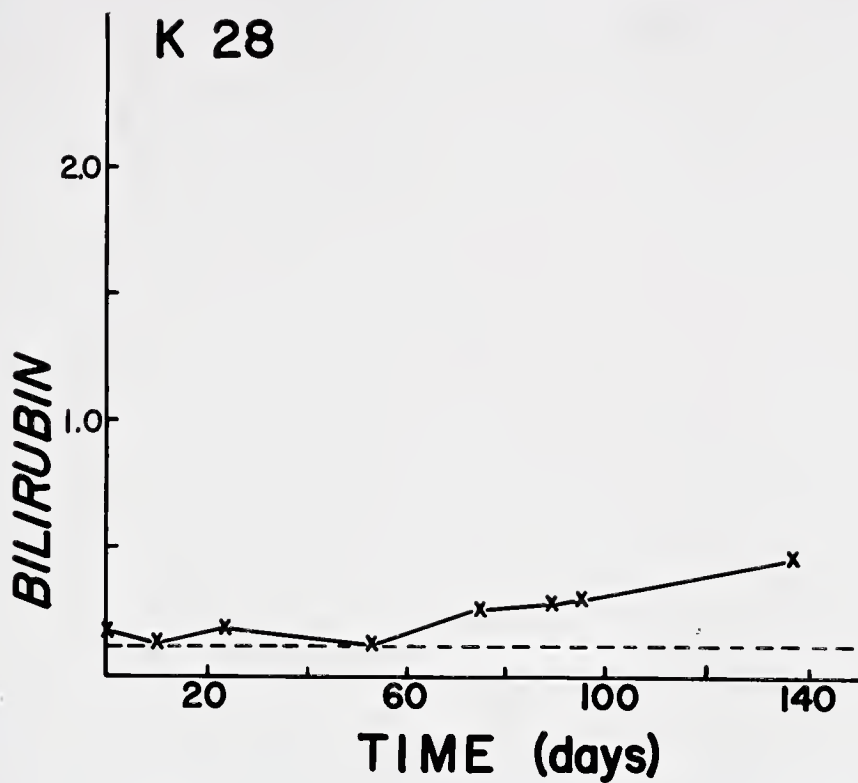


Fig. 65

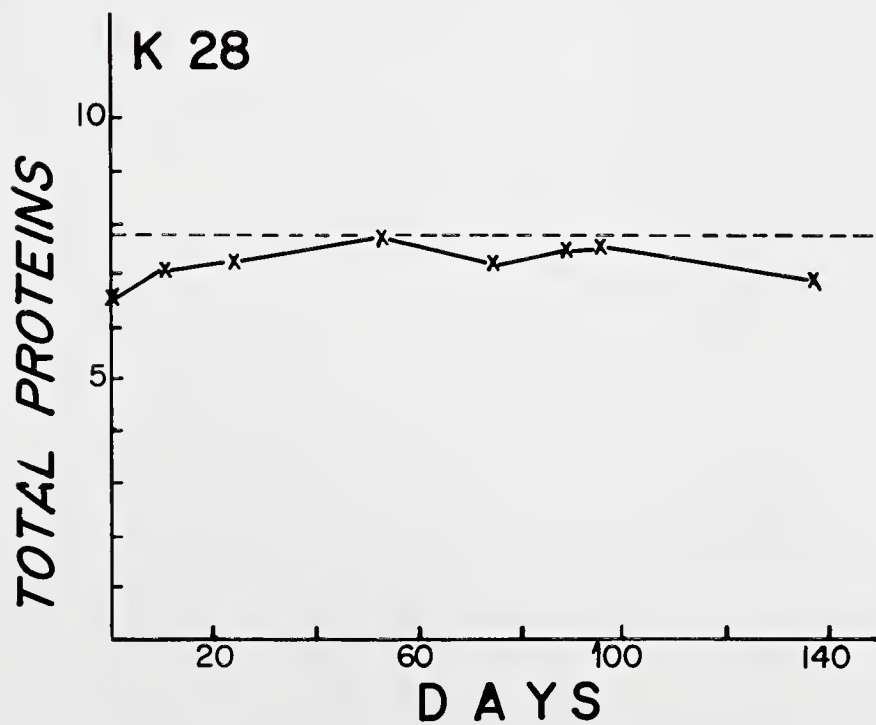


Fig. 66

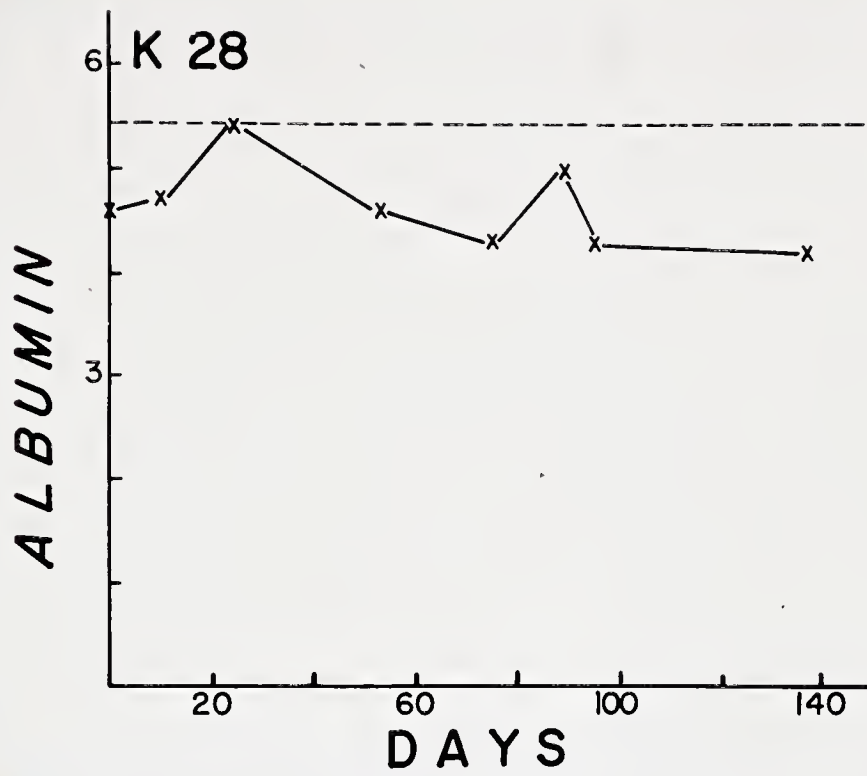


Fig. 67

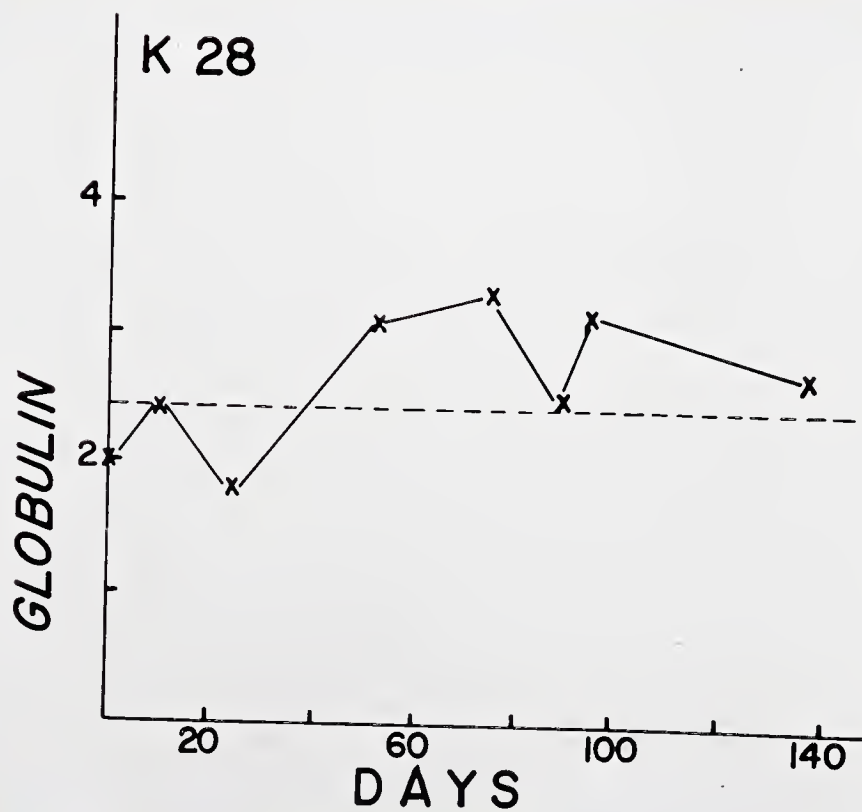


Fig. 68

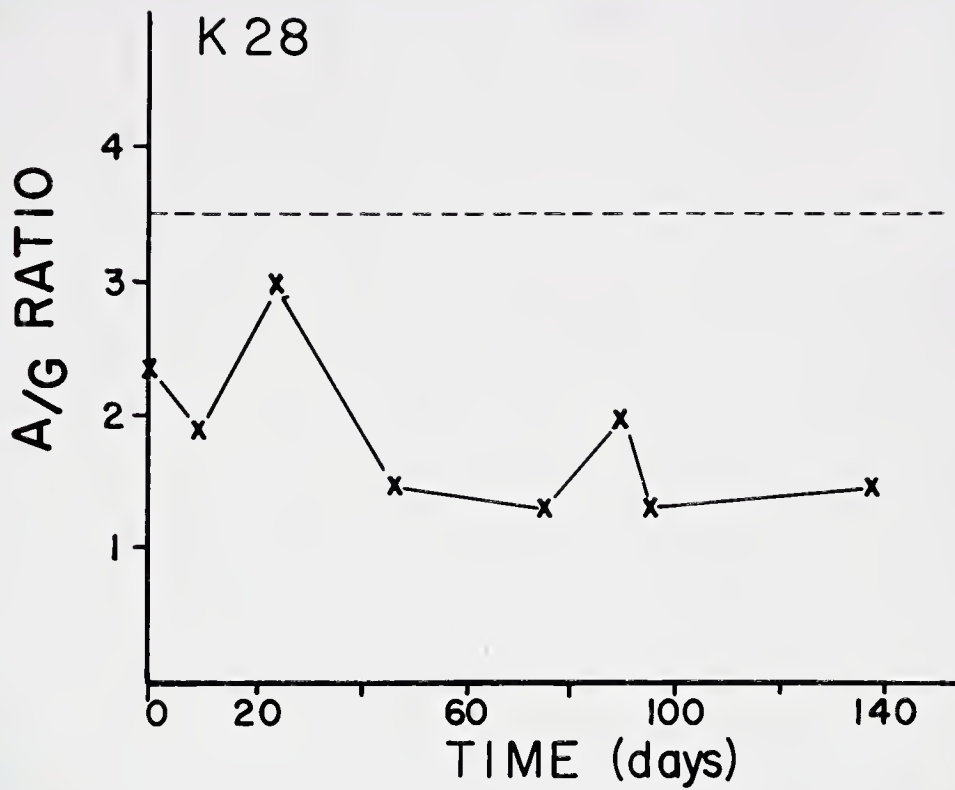


Fig. 69

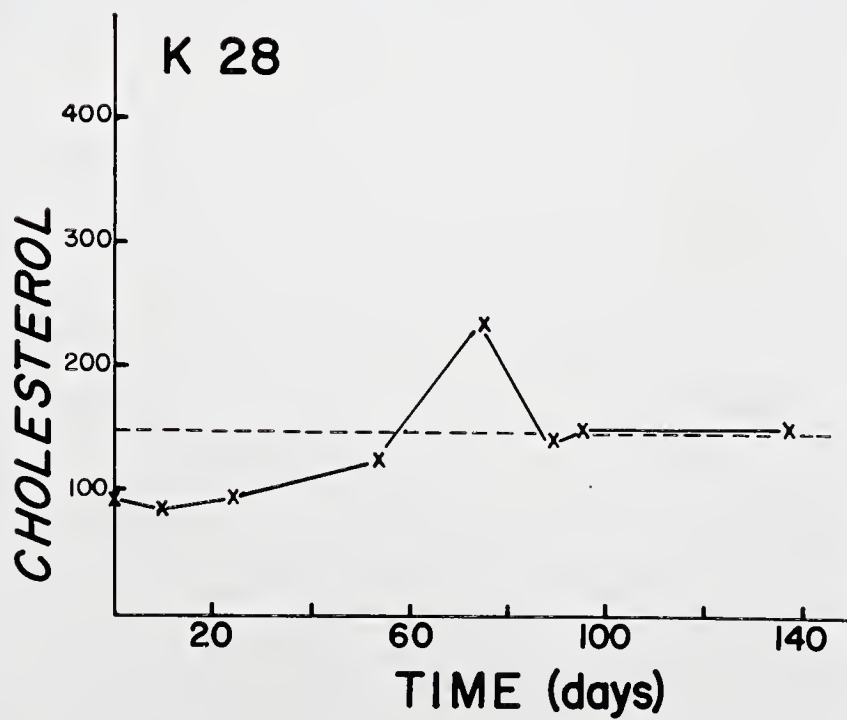


Fig. 70

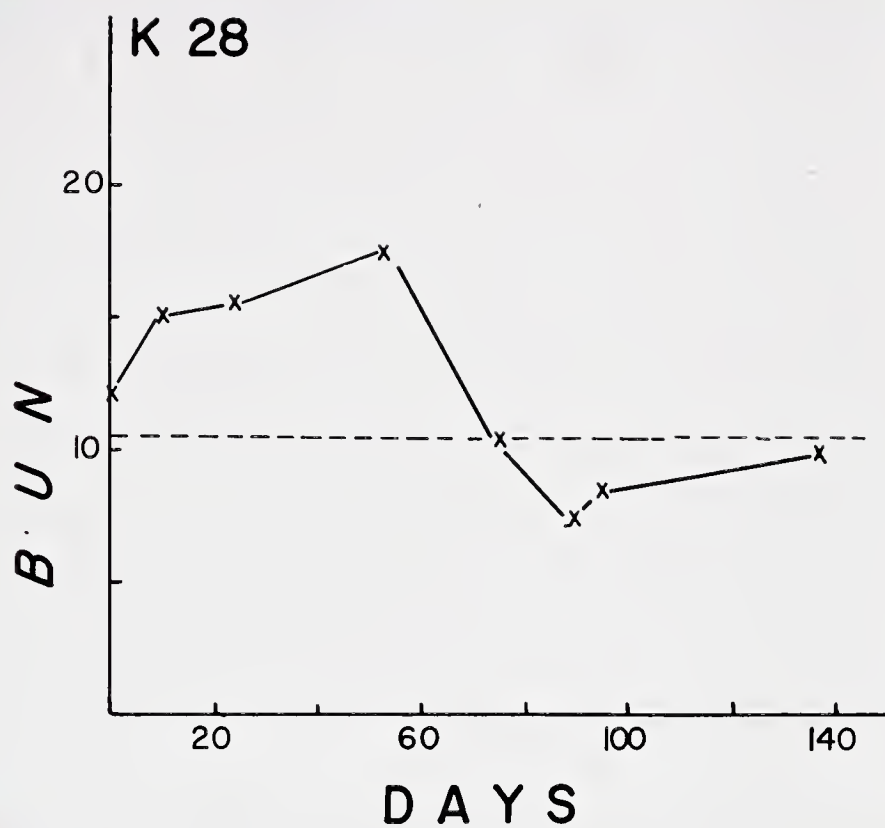


Fig. 71

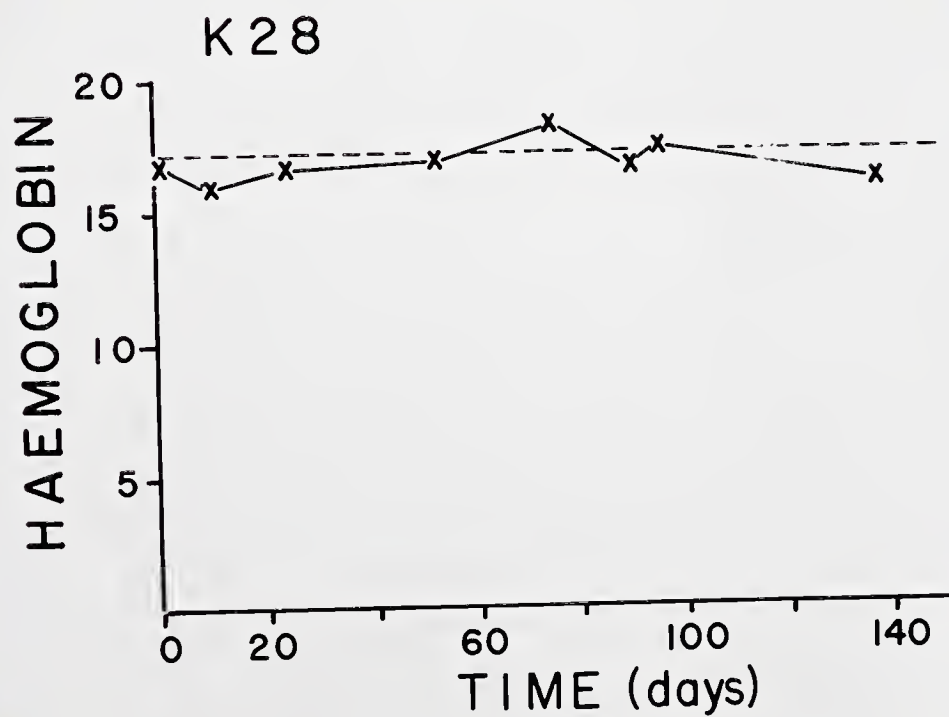


Fig. 72

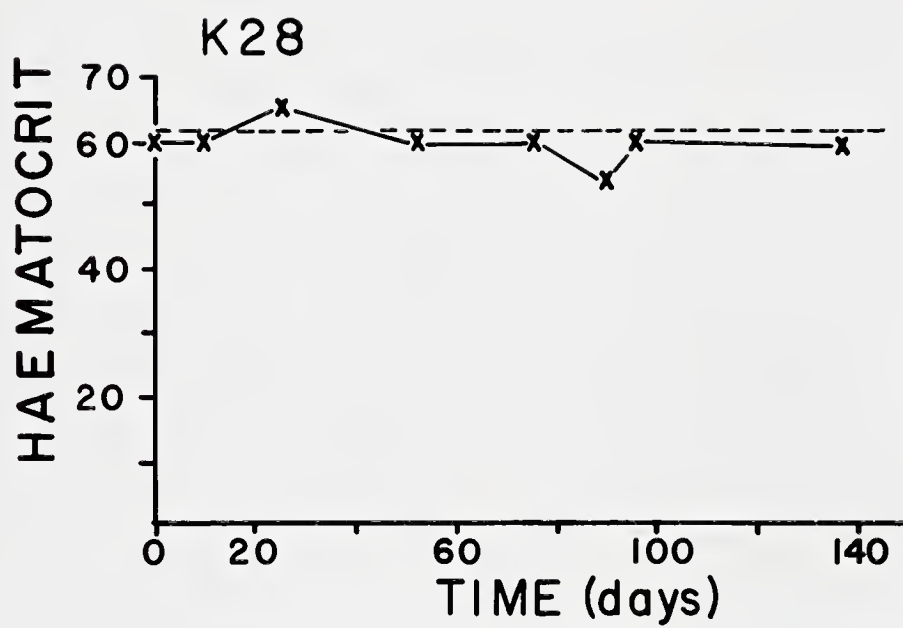


Fig. 73

DOG K-29

Hematological and biochemical investigation on the 25th of November, 1959, revealed no abnormality of liver function. Clinically the animal was alert, fit and healthy. Oral feeding with carbon tetrachloride in gelatin capsules commenced on the 27th of November, 1959. Initially the animal was given 1.5 ml. carbon tetrachloride every second to third day. This was gradually increased until at the time of demise, 110 days after the start of the experiment the dog was receiving 5 ml. carbon tetrachloride on alternate days. Death occurred on the 17th of March, 1960, three days after 15 ml. carbon tetrachloride had been introduced into the dog's stomach by means of a nasogastric tube. This procedure was performed under general anaesthesia induced by the administration of Nembutal intravenously. The dosage used was 0.5 ml. Nembutal per kilogram body weight. The dog never regained consciousness and succumbed during the morning of the 17th of March, 1960. The cause of death was presumed to be due to Barbiturate poisoning in view of the diseased liver which was unable to detoxify the Nembutal. Measures were taken to counteract this intoxication including the administr-

ation of Megimide and Picrotoxin intravenously as well as an intravenous transfusion of 5% Dextrose in water, but to no avail.

During the period of the experiment, 110 days in this instance, 159.25 ml. carbon tetrachloride were administered to the animal. The animal's weight decreased from 20 kg. initially to 16 kg. at the time of the induction of the fatal anaesthetic. The dog appeared clinically well and healthy until this time.

A postmortem examination performed shortly after the animal's demise revealed the following findings. The liver was small, mottled and light brown in color. Its surface was finely nodular and its consistence was rubbery. On biopsy the liver incised coarsely. There was no evidence of an increased portal systemic collateral circulation. The spleen appeared normal. Ascites was not present. The lungs were found to be hemorrhagic and edematous.

HISTOPATHOLOGY

This specimen demonstrates well the criteria necessary to confirm the diagnosis of cirrhosis, e.g.

(1) Disorganization of hepatic architecture (2) Increase in connective tissue (3) Nodular regeneration (4) Hepatocellular degeneration (5) Bile duct proliferation.

The type of disorganization of the lobular

pattern is exceedingly diffuse, produced by a gross increase in the amount of connective tissue which has spread irregularly throughout the liver substance. It has divided the lobules into areas of varying sizes in which regeneration is evident by virtue of (1) Increase in cell plate thickness (2) Increase in size of cells (3) Increase in mitotic figures.

In this case, in the absence of special connective tissue stains, it is difficult to differentiate between "active" and "passive" connective tissue, and it seems that much of the so-called connective tissue is in fact compression of cellular framework due to postnecrotic collapse.

Although the lobular architecture is heavily obscured it can still be recognized. The "increase" in connective tissue appears to have resulted in many areas from centrilobular cellular collapse, an increase which may be said to be passive. However, there are numerous finger-like processes of connective tissue joining centrilobular areas to portal tracts as well as other centrilobular areas, which have subdivided the parenchyma into nodules and pseudolobules, which have themselves been further subdivided by interdigitations of connective tissue septums.

In this specimen the increase in connective tissue may be said to be (1) Centrilobular (2) Intraparenchymal (3) Perilobular (4) Perinodular (5) Portal and (6) Periportal. This is a combination of a diffuse increase of

both "active" and "passive" connective tissue, in association with a diffuse small nodular regeneration. A diagnosis of a mixed cirrhosis, with evidence of both portal and postnecrotic types seems justified, for in some areas the cellular necrosis has been fairly widespread resulting in almost complete lobular collapse, producing rather broad sheets of reticulum staining material, due presumably to collapse.

This animal was particularly resistant to the control of necrosis by the use of SGOT monitoring. One wonders if this may not be due to some form of hepatocellular sensitivity responsible for this diffuse increase in marked necrosis and collapse in an animal subjected to only 159 ml. carbon tetrachloride enterally over a period of 110 days.

In many the cells of the centrolobular zones are destroyed by severe hemorrhage. This may be accounted for by an agonal centrolobular necrosis and hemorrhage as a result of death due to cardiac failure following the gastric intubation of 15 ml. carbon tetrachloride under Nembutal anesthesia.

There is a widespread bile duct proliferation noted particularly in the portal and periportal tracts and also in the numerous connective tissue sheets traversing the lobular structures. Also present in relation to the connective tissue is an increase in inflammatory cells and

histiocytes which occur in close association with the proliferating bile ducts. The cells are mainly of the monocytic variety. The proliferating bile ducts are in many instances surrounded by connective tissue in which young fibroblasts are evident. Numerous giant cells and histiocytes are seen enmeshed in the connective tissue.

The reticulum stain demonstrates clearly the diffuse fragmentation of the lobular pattern, as well as the form of the actively regenerating nodules with "passive" septums at their periphery.

It was also noted in this specimen, how irregularly the hepatic vasculature had been disturbed and in particular the manner in which central veins were aggregated and collapsed. (See Figs. 74-77)

BIOCHEMISTRY

In this animal there was great difficulty in maintaining the SGOT level below 100 units, even while using small doses of carbon tetrachloride. Certainly in this instance, and with some of the other experimental animals in this project, it was noticed that the reaction to carbon tetrachloride as judged by SGOT estimations was quite unpredictable.

The BSP retention was closely correlated with the SGOT levels, the relationship being directly proportional. It rose early in the experiment and remained pathologically elevated.

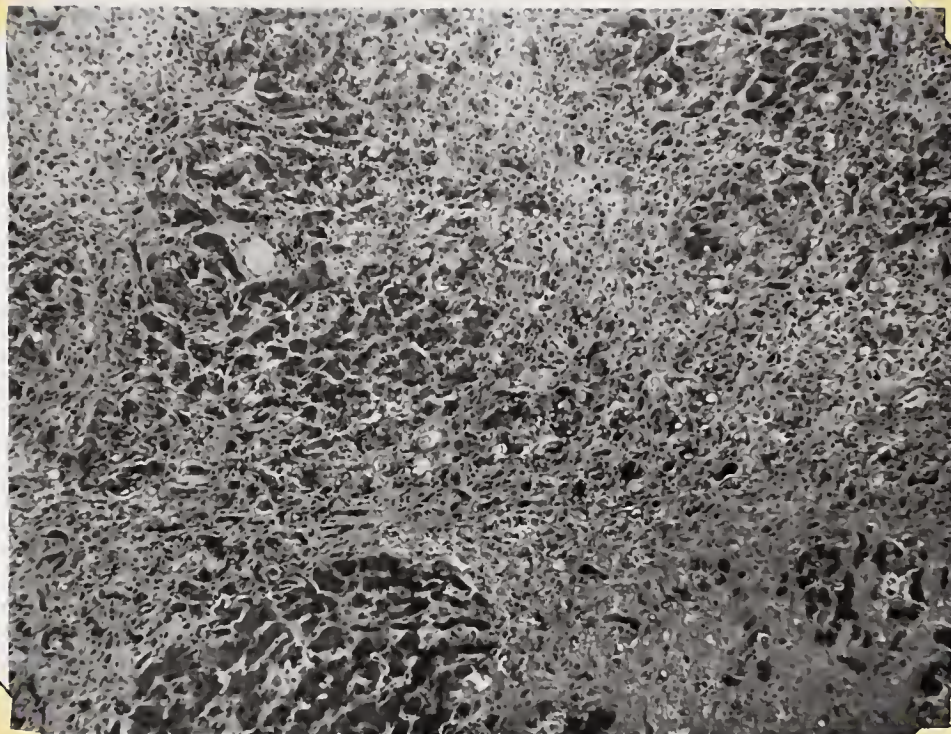


Fig. 74

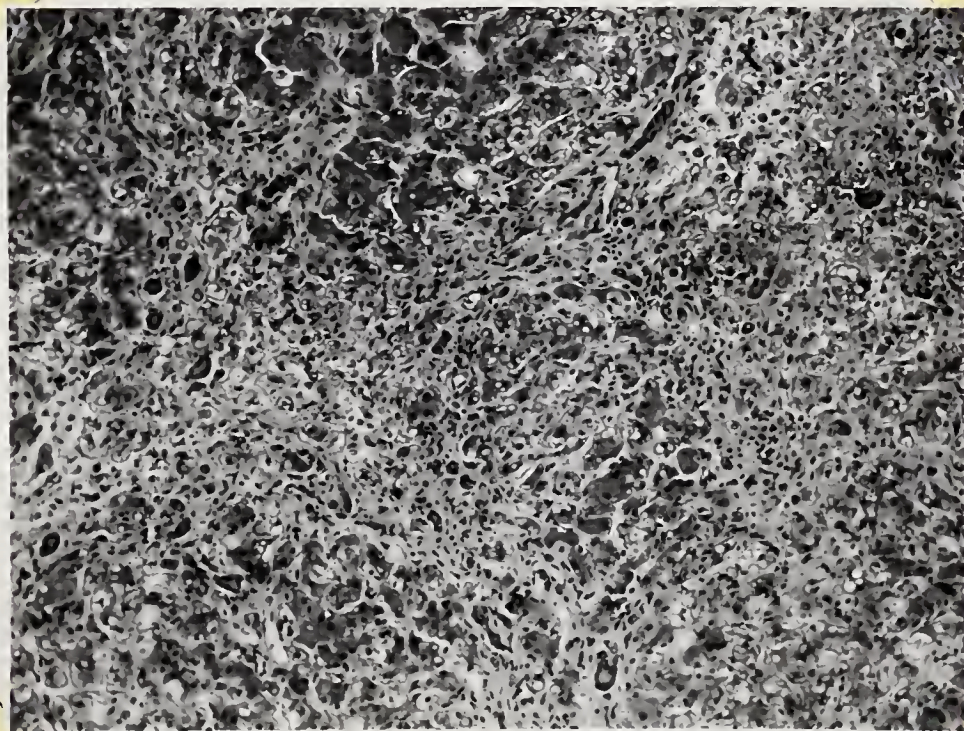


Fig. 75

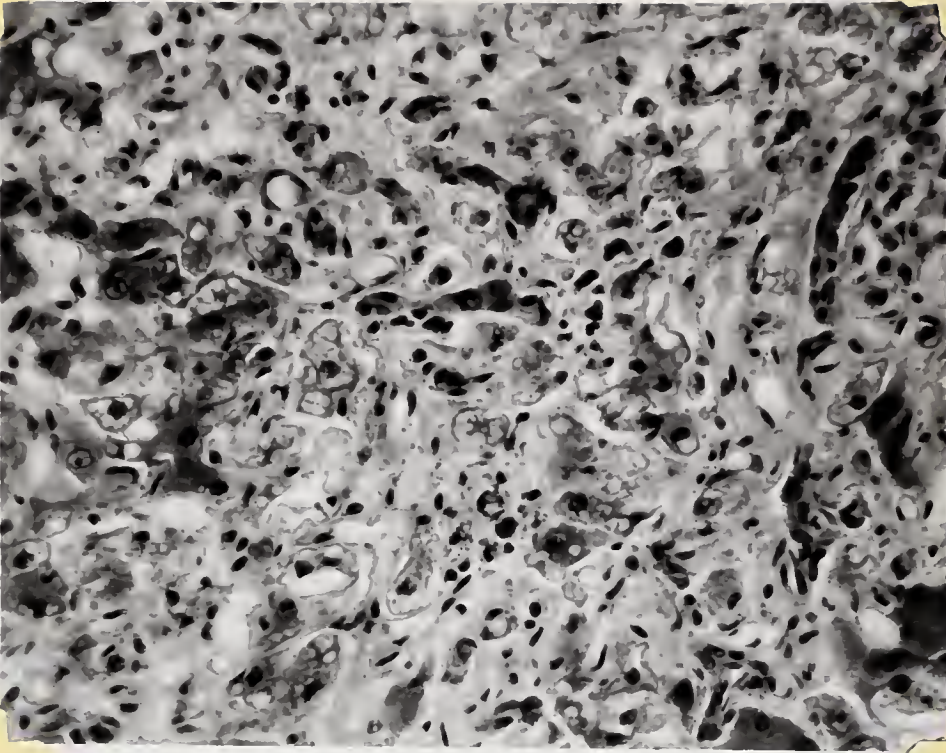


Fig. 76

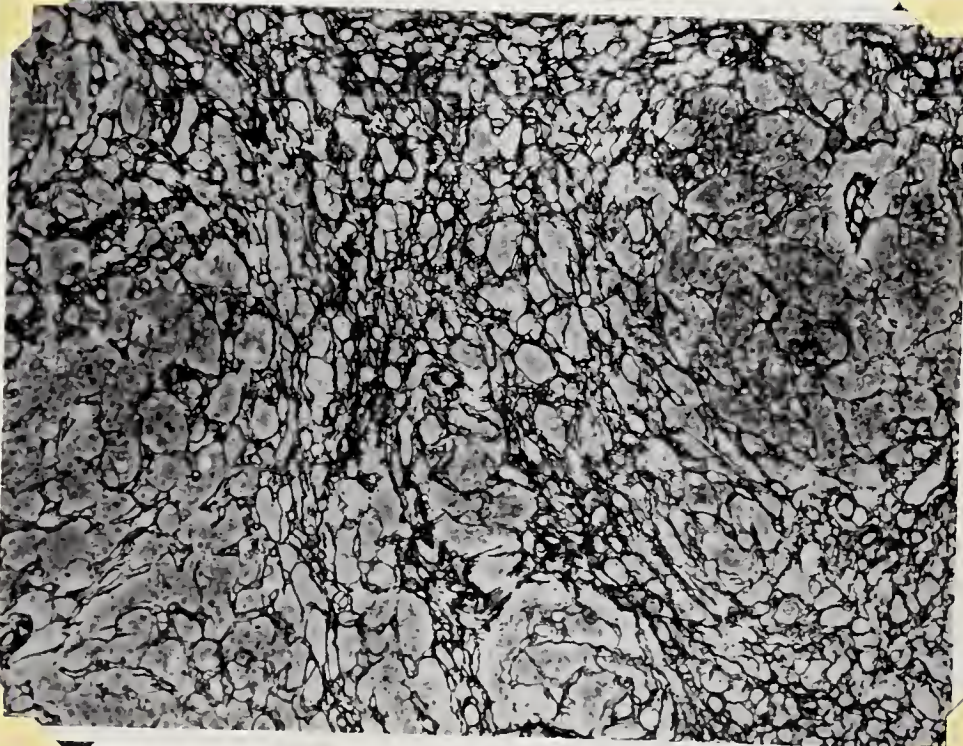


Fig. 77

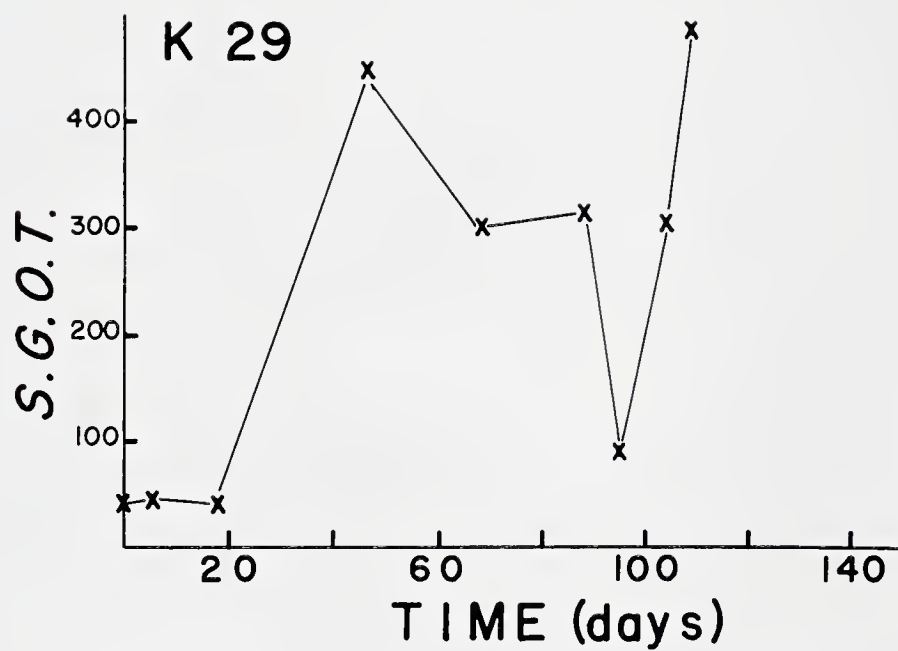


Fig. 78

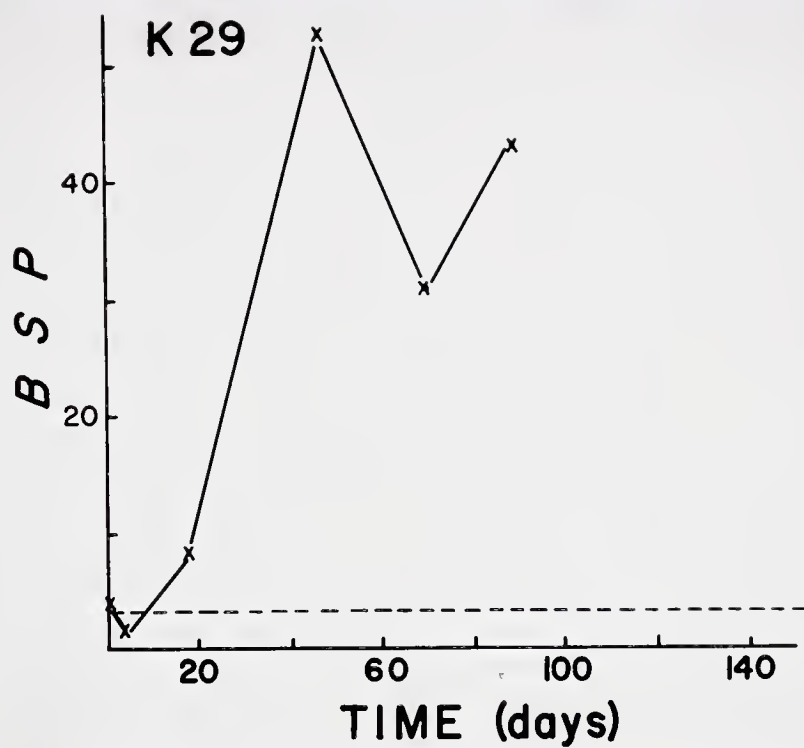


Fig. 79

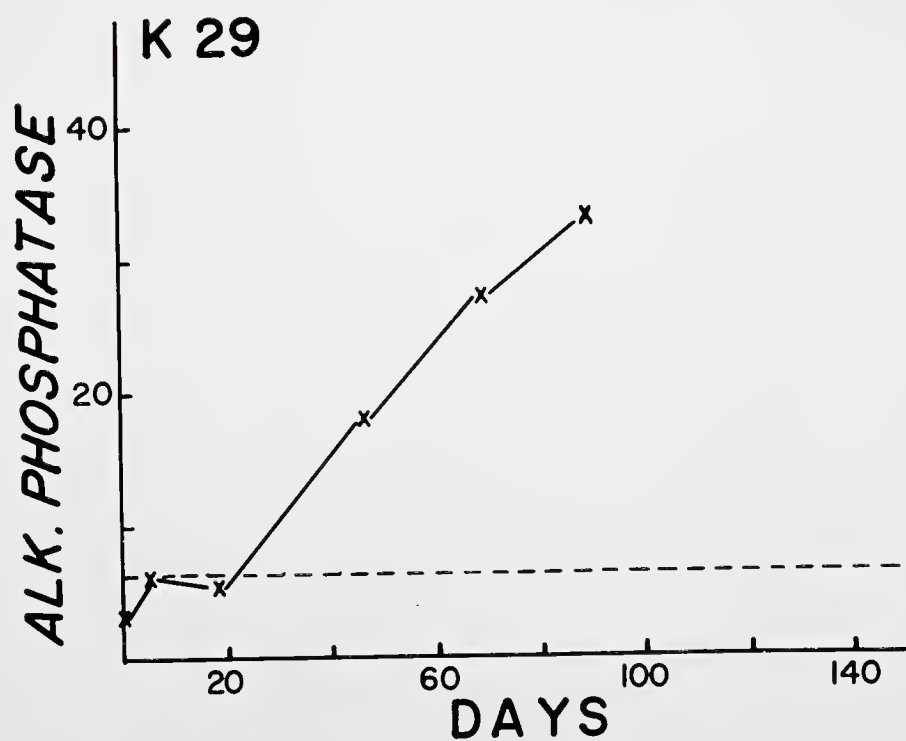


Fig. 80

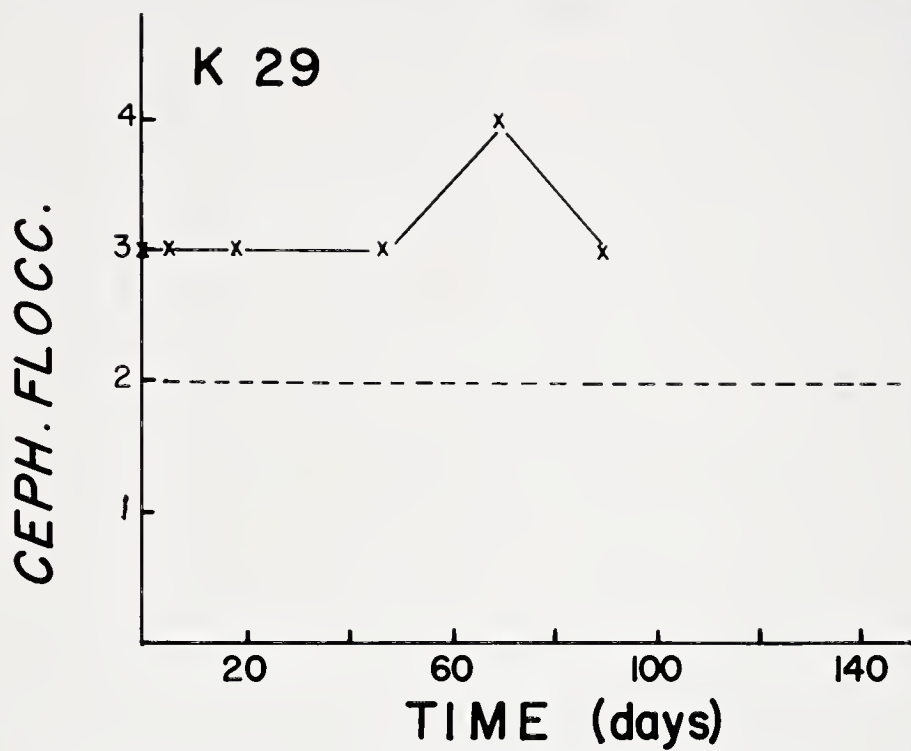


Fig. 81

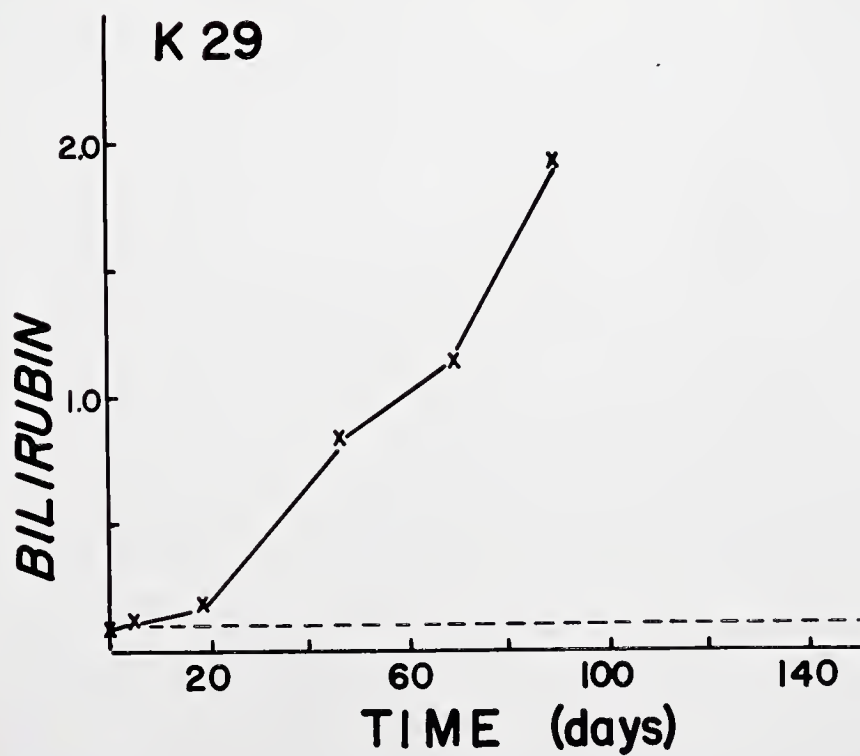


Fig. 82

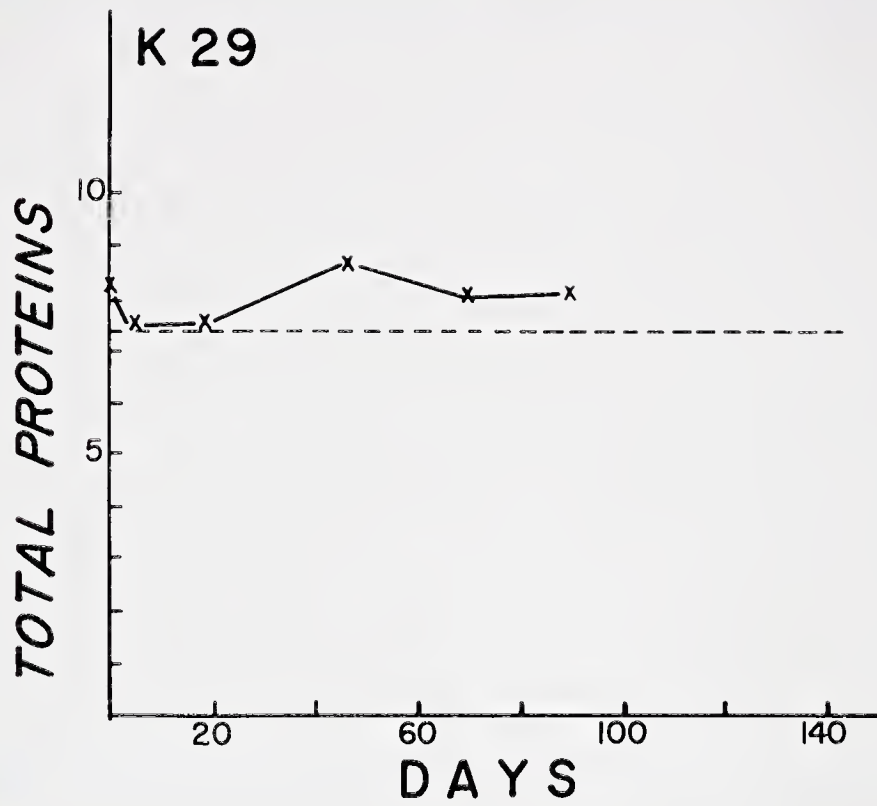


Fig. 83

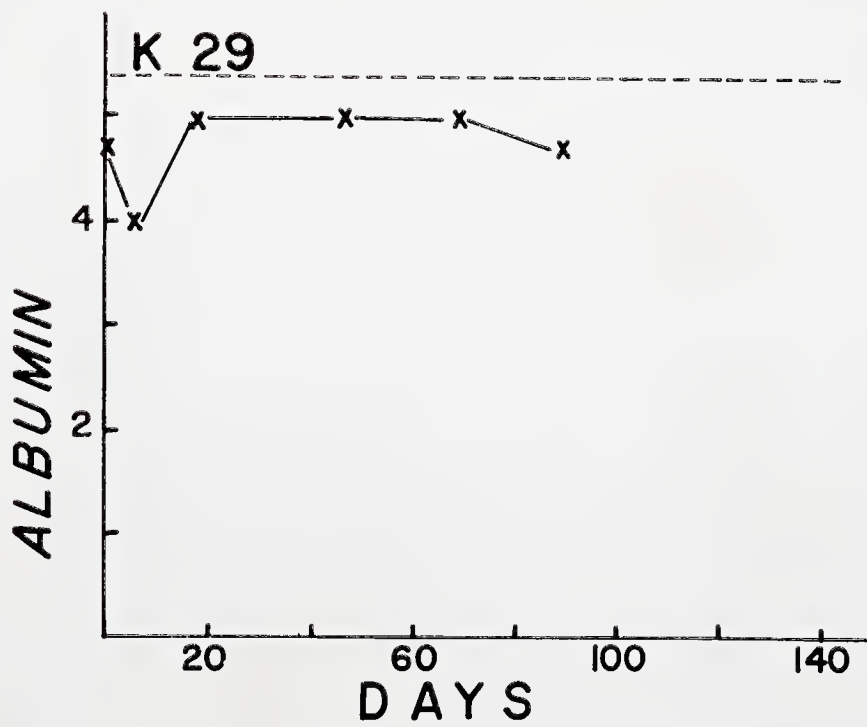


Fig. 84

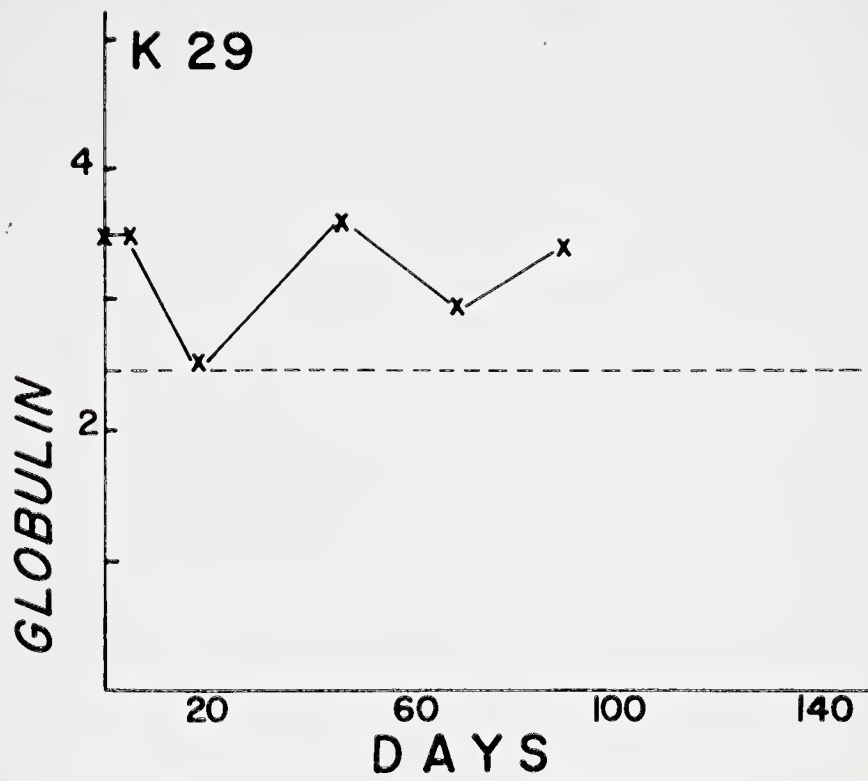


Fig. 85

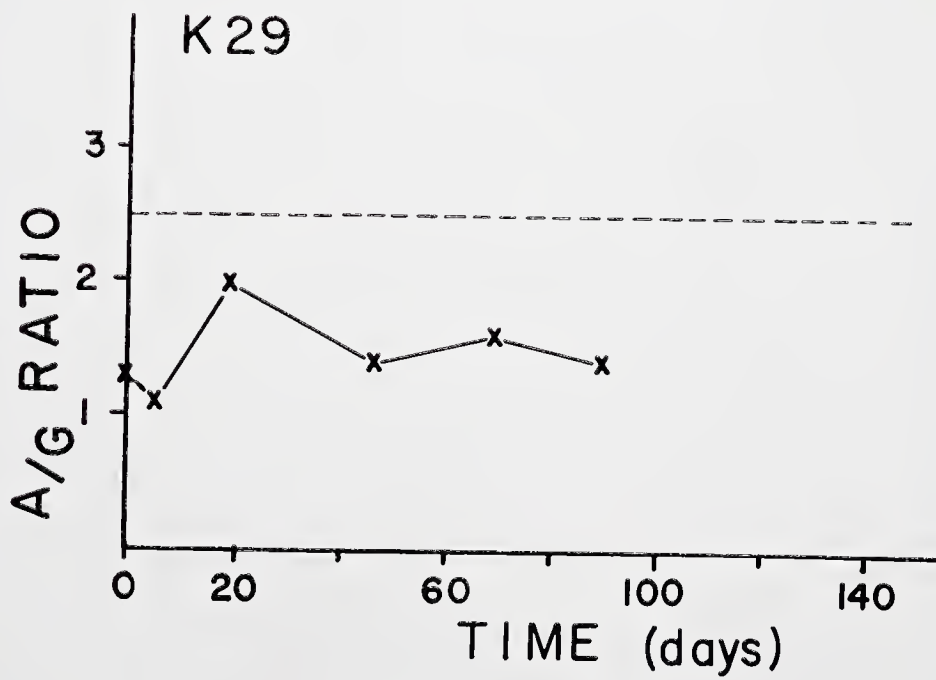


Fig. 86

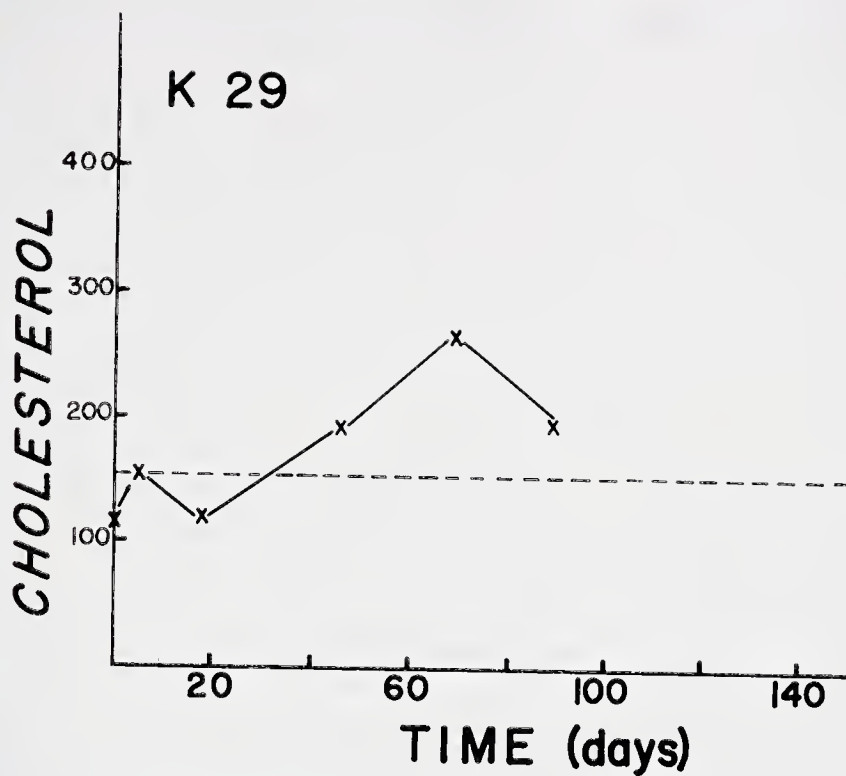


Fig. 87

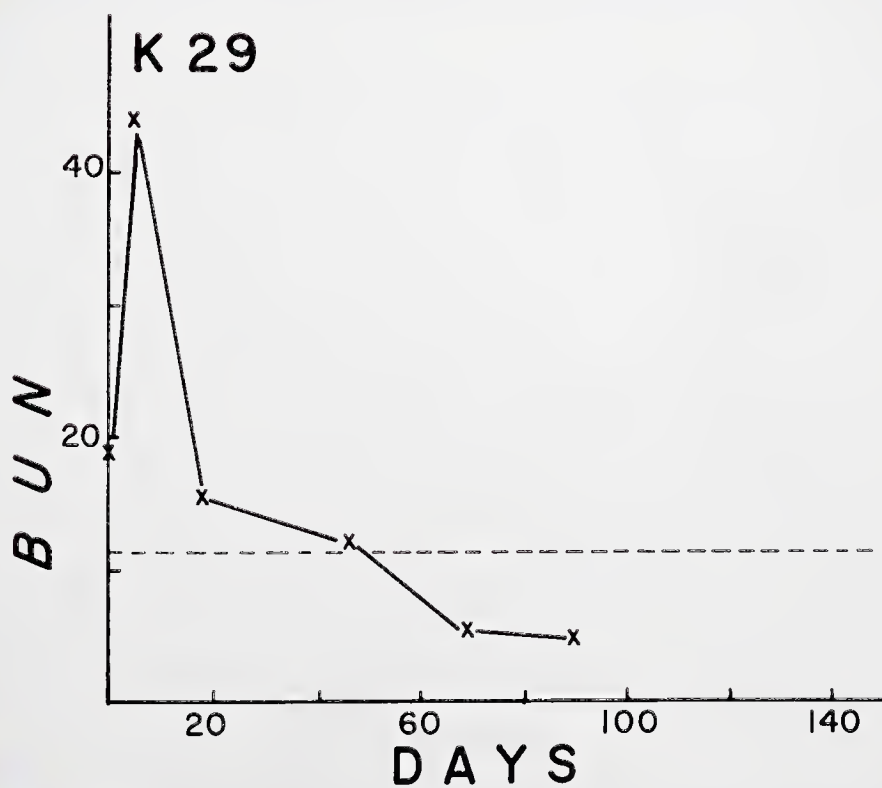


Fig. 88

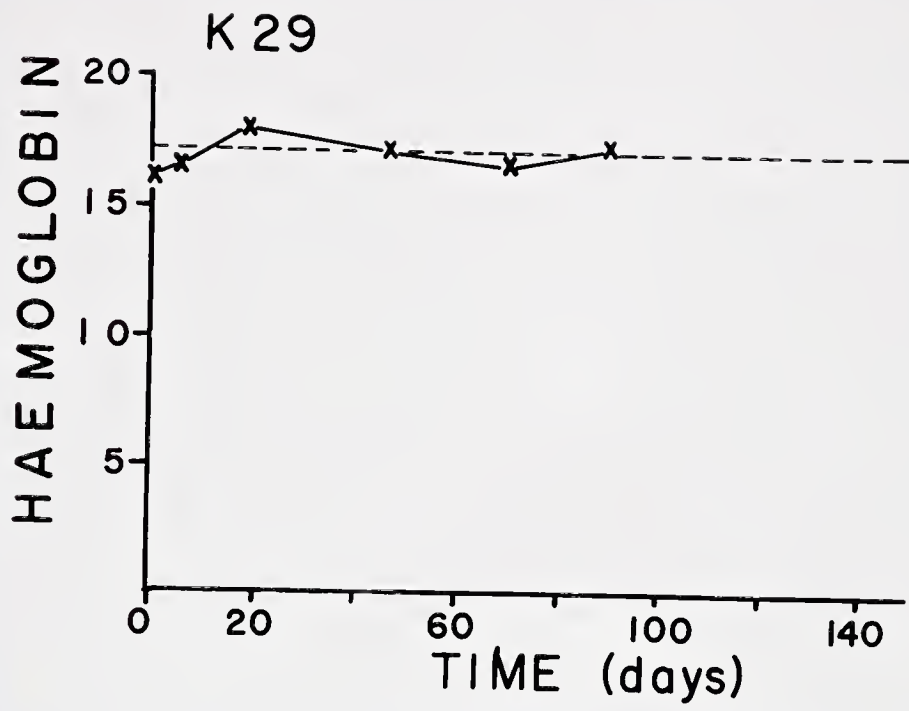


Fig. 89

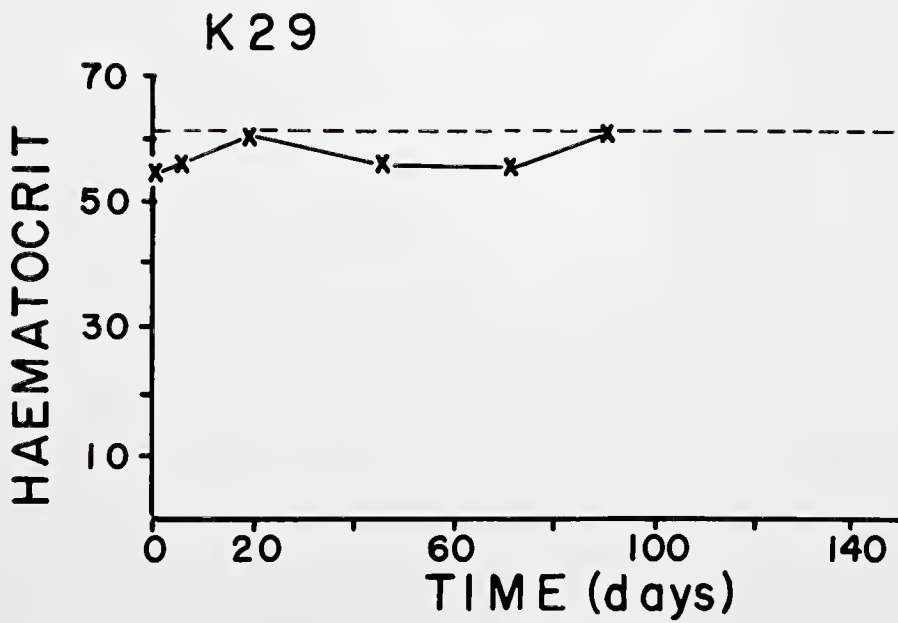


Fig. 90

DOG K-30

Hematological and biochemical investigation on the 7th of December, 1959, revealed no abnormality of liver function. Clinically the animal was alert, fit and healthy. Oral feeding with carbon tetrachloride in gelatin capsules commenced on the 8th of December, 1959. Initially the animal was given 1.5 ml. carbon tetrachloride every second to third day. This was gradually increased until at the time of laparotomy 99 days after the start of the experiment, 10 ml. carbon tetrachloride was being administered on alternate days. The dose of carbon tetrachloride was regulated by the level of SGOT. The aim was to keep the SGOT level below 100 units so that gross necrosis would be prevented. On several occasions the SGOT level rose well above this level, although the dose of carbon tetrachloride had not been increased. Whether this was due to sensitivity to the drug, the accumulative effect, or some other cause was not determined. However, the dog remained clinically well throughout the experiment. Its initial weight was 14.5 kg. and its weight at the time of laparotomy 99 days later was 13 kg. Over this period the animal received 270 ml. carbon tetrachloride via the oral route.

FINDINGS:

The liver appeared smaller than normal and was of a pale, yellowish color. The surface exhibited a regular nodularity, the nodules being small, measuring some 2 - 3 mm. in diameter. The consistency of this organ was firm to rubbery, a finding which was confirmed on incisional biopsy. The spleen appeared normal and there was no evidence of a portal systemic collateral circulation or ascites. No other abnormality was noted. The portal pressure measured 320 mm. saline.

HISTOPATHOLOGY

The Hematoxylin and Eosin, and Reticulum staining techniques ably demonstrate a diffuse cirrhosis of the septal or portal variety in this specimen.

The normal lobular pattern is largely obscured, being recognizable in few areas. The normal pattern is replaced by a diffuse nodular hepatocellular hyperplasia associated with an equally diffuse increase in connective tissue. The Hematoxylin and Eosin stain demonstrates this as sheets and cords of fibroblasts irregularly dividing up the liver substance and frequently containing numerous proliferating bile ducts. In some regions, particularly at the periphery of regenerative nodules, the increase in connective tissue has the appearance of

compressed degenerative and necrosed cells as well as occasional fibroblasts and monocytes. There is also a generalized increase in the presence of inflammatory cells, mainly of the monocytic variety. Numerous histiocytes are seen.

Few central veins are visualized and of those that are seen, many are collapsed or disturbed by the diffuse regeneration and fibrosis. In many instances the portal tracts are distorted as well.

The reticulum stain demonstrated mainly a diffuse septal increase in reticulum with diffuse nodule formation. "Passive" compressed septums were present at the periphery of the nodules and particularly between contiguous nodules. In a few areas, broad bands of reticulum were suggestive of a postnecrotic type of collapse, while in others the appearance was compatible with simple centro-lobular necrosis.

This may be construed as evidence that various forms of degeneration, necrosis, fibrosis and regeneration are proceeding simultaneously and that the end-result will vary with the different pathways taken by these pathological processes.

The high portal pressure in this case is in keeping with the histopathological picture which demonstrates clearly a marked distortion and compression of the hepatic vasculature, particularly affecting the central veins. (See Figs. 91-94)

BIOCHEMISTRY

With the progress of the experiment the liver function tests generally demonstrated a diminishing ability of the liver to function adequately. The greatest diminution of function was observed to coincide with that period during which the SGOT levels were markedly raised. The Serum Alkaline Phosphatase, BSP retention and Serum Bilirubin levels rose steeply during the first two months and then descended but remained above the average normal levels. The Cephalin Cholesterol flocculation remained unchanged for the first 30 days, then rose and remained elevated for the remainder of the experiment. The Serum Cholesterol level rose initially but soon returned to an average normal level where it remained.

The BUN level fluctuated around the average normal level.

The Total Serum Protein level rose slightly during the first 30 days then descended to below the average normal level during the subsequent 30 days, after which it gradually rose to a level slightly above the pre-experimental reading. The Serum Albumin level gradually descended while the Serum Globulin level, after some initial fluctuation, rose to well above its

initial level. The Albumin-Globulin ratio exhibited a gradual decrease until it was reversed. Even in the short period of 99 days there was evidence of a constant gradual impairment of liver function, although the animal remained clinically fit and well. (See Figs. 95 - 105)

HEMATOLOGY

Both Hemoglobin and Hematocrit levels descended slightly over the first half of the experimental period then gradually rose again. At all times, however, the readings were within the limits of normality. See Figs. 106, 107).



Fig. 91

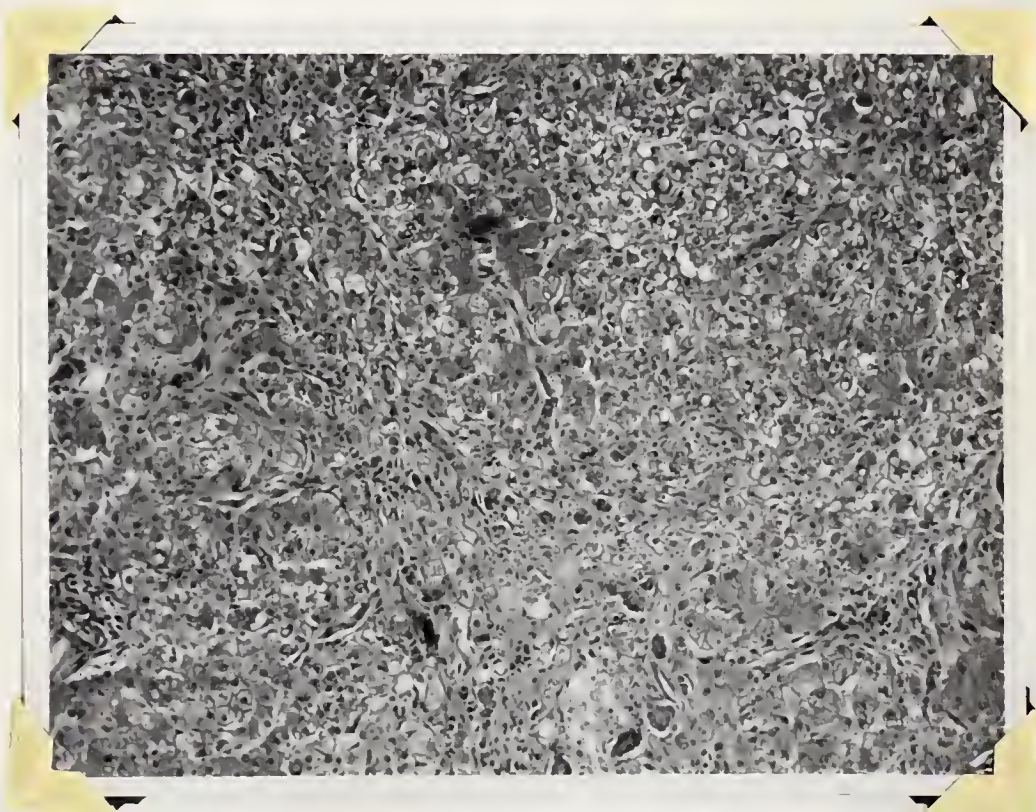


Fig. 92

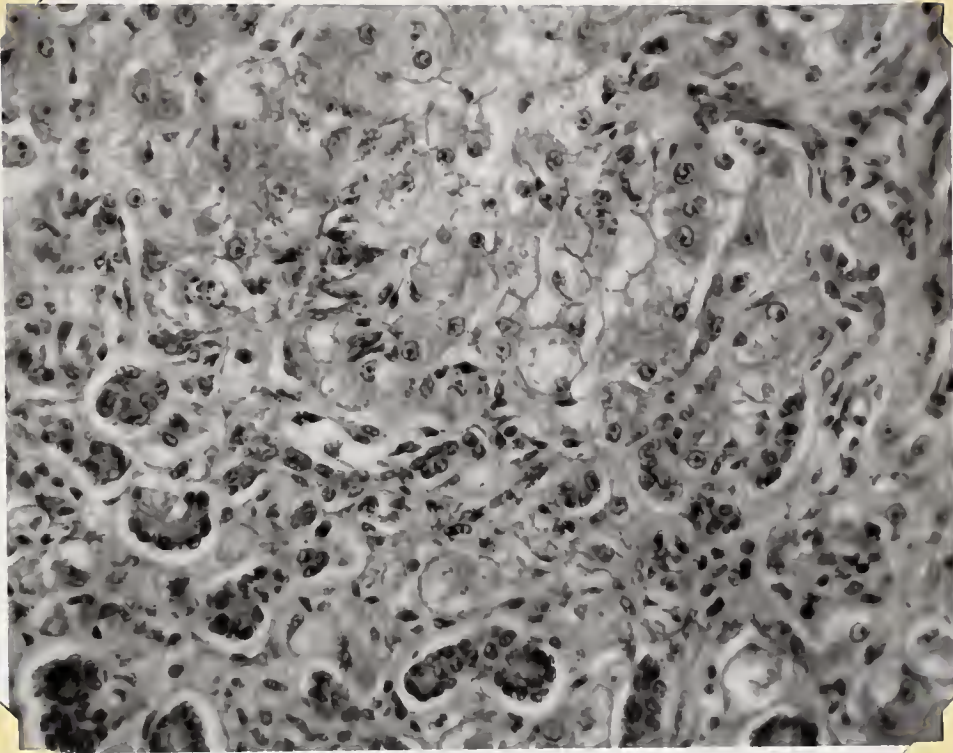


Fig. 93

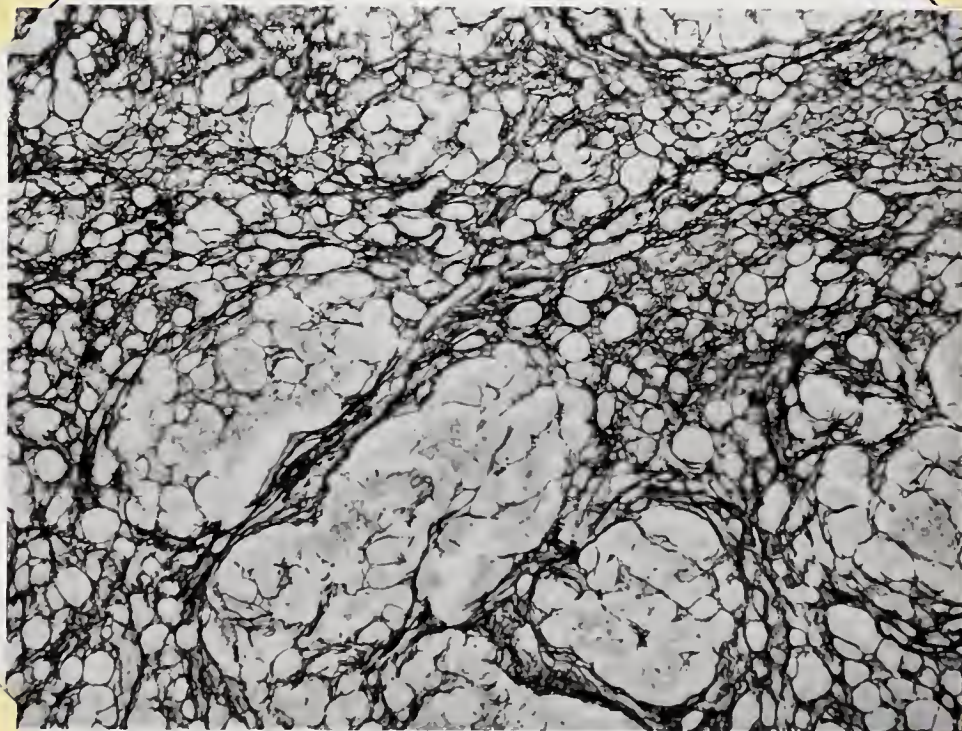


Fig. 94

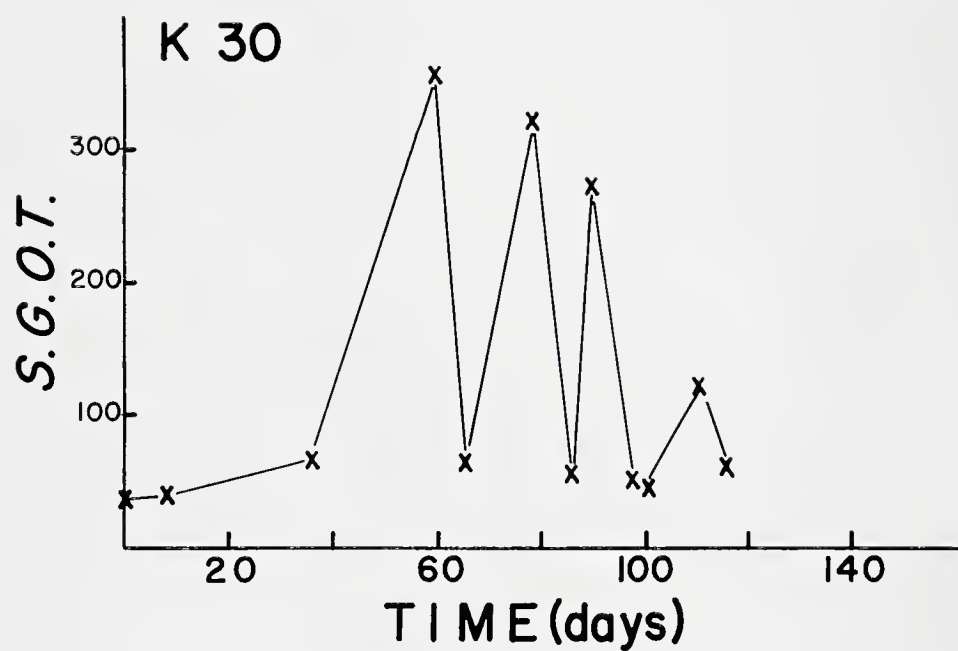


Fig. 95

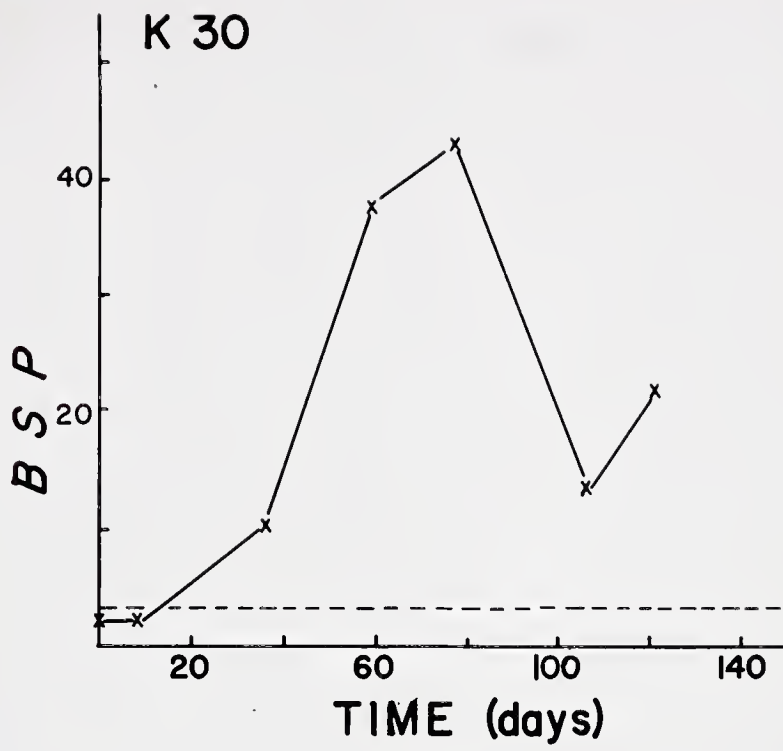


Fig. 96

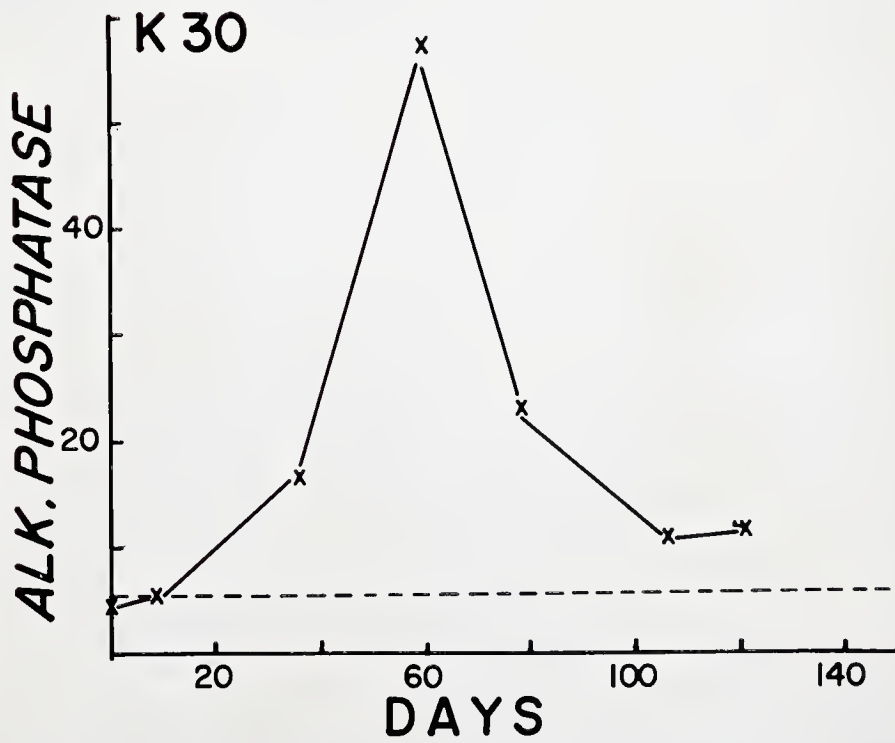


Fig. 97

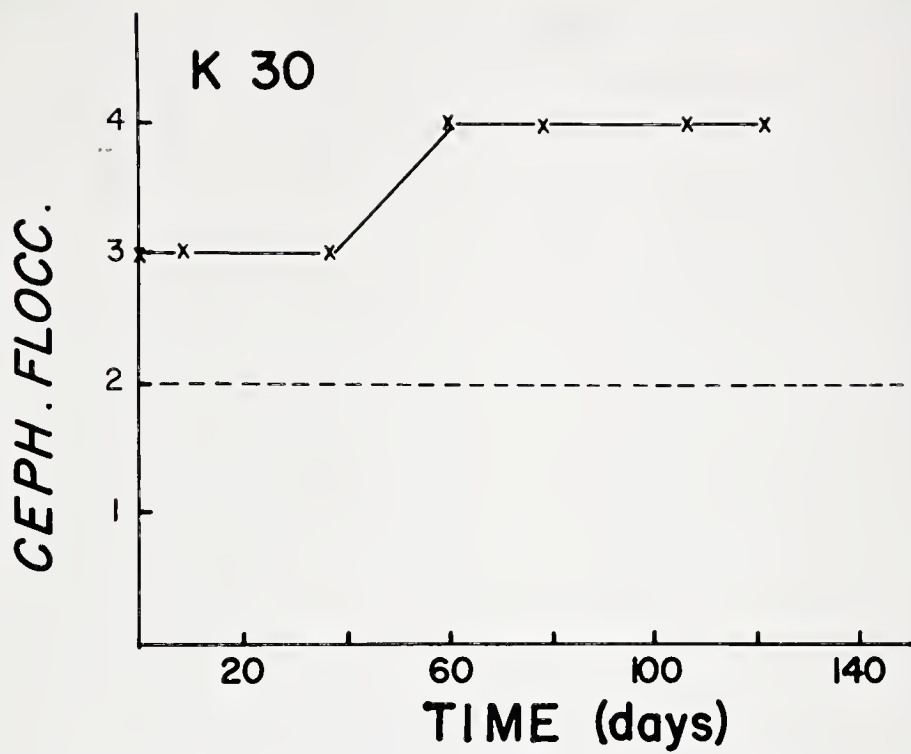


Fig. 98

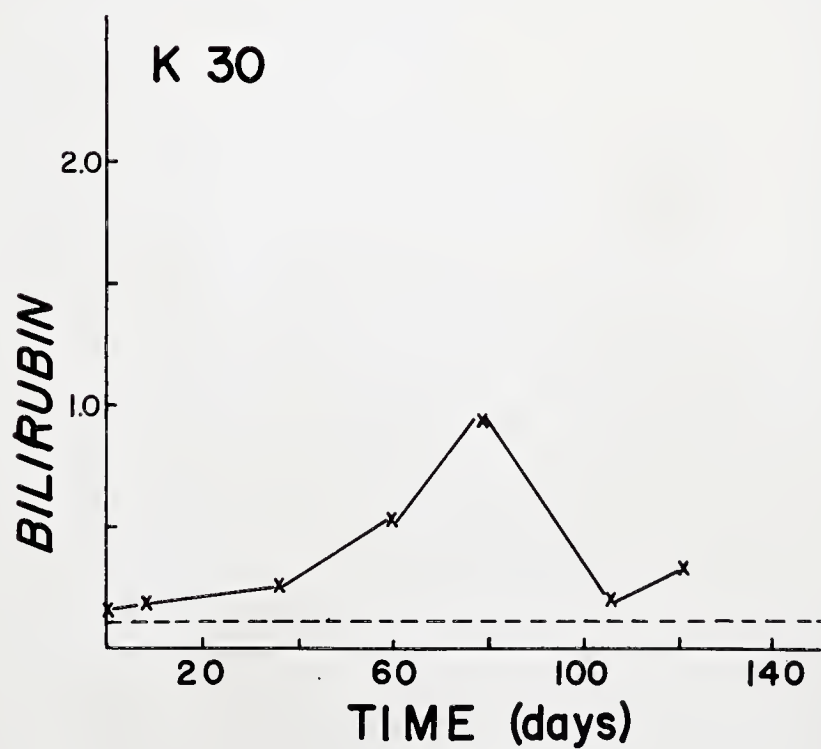


Fig. 99

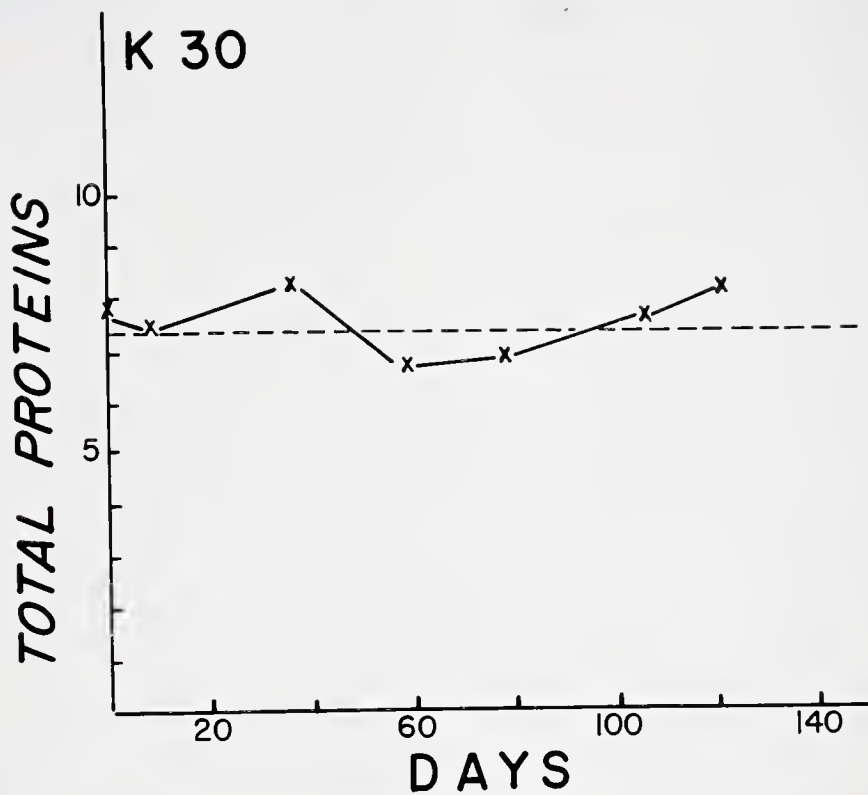


Fig. 100

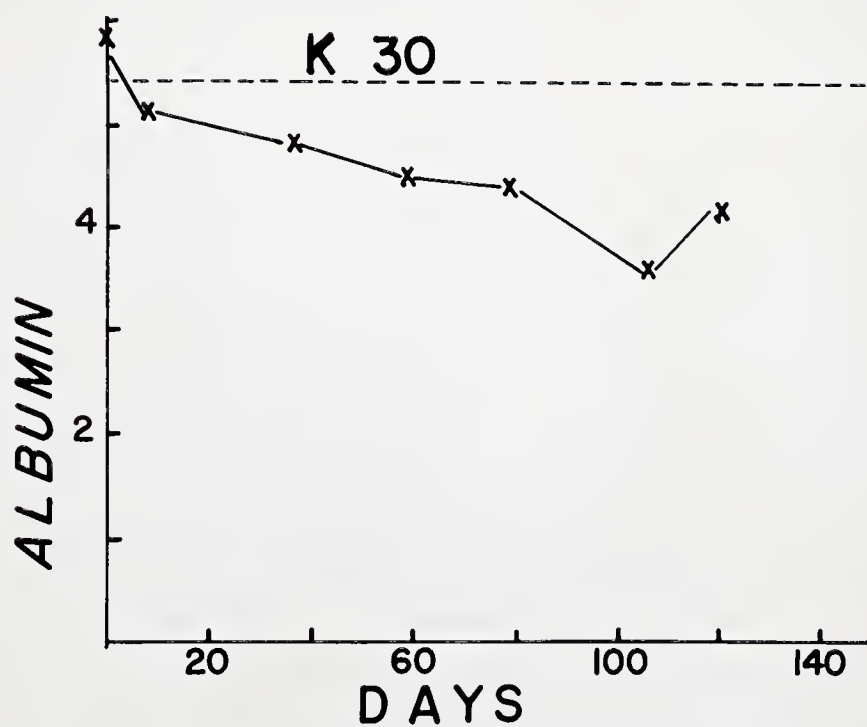


Fig. 101

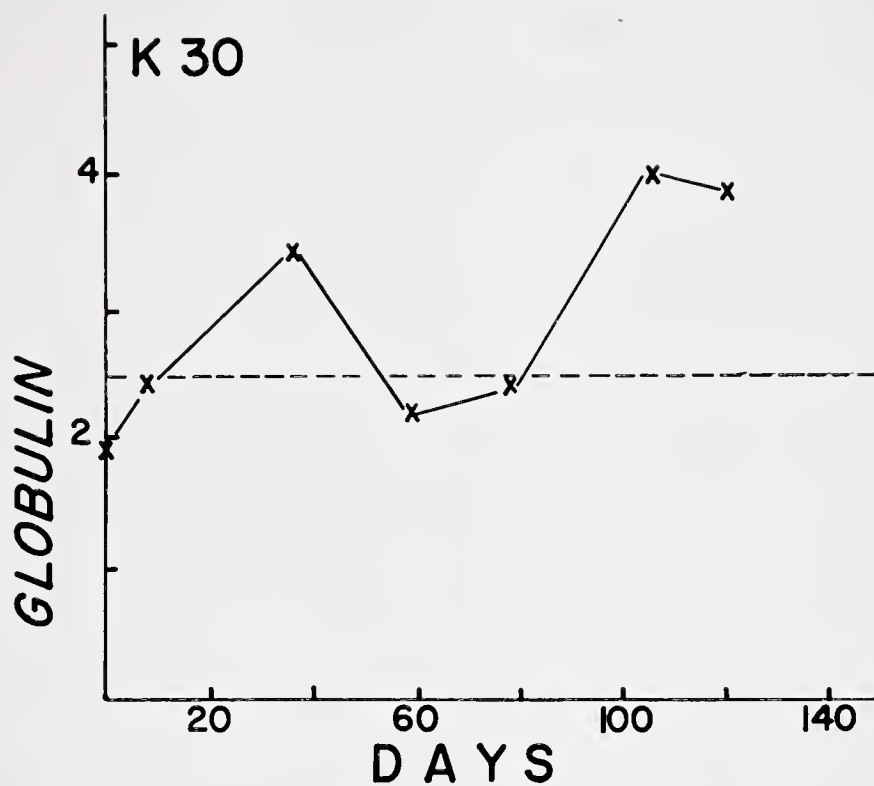


Fig. 102

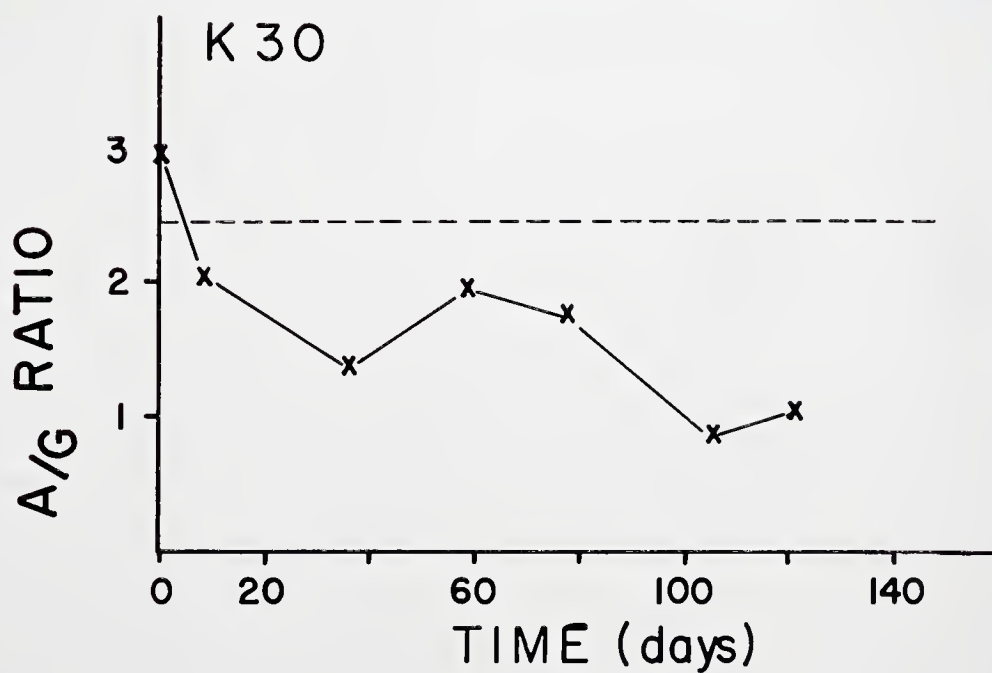


Fig. 103

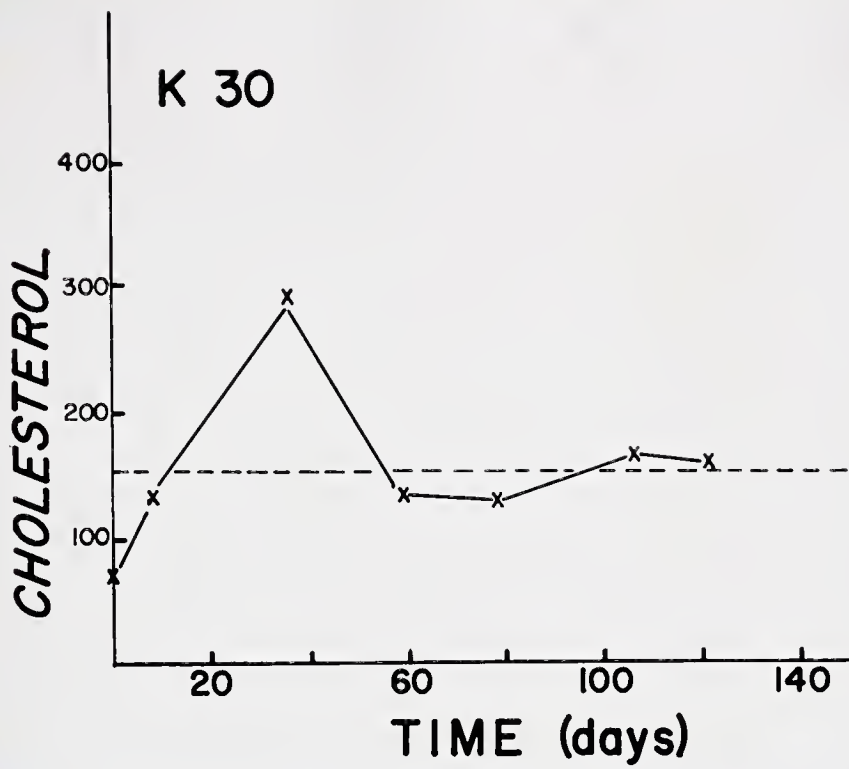


Fig. 104

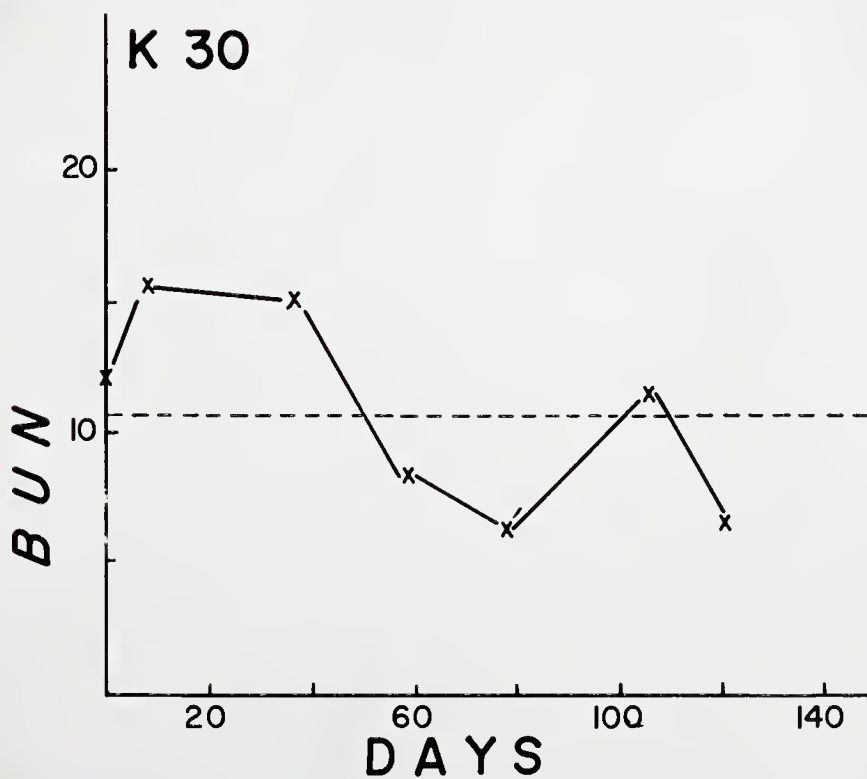


Fig. 105

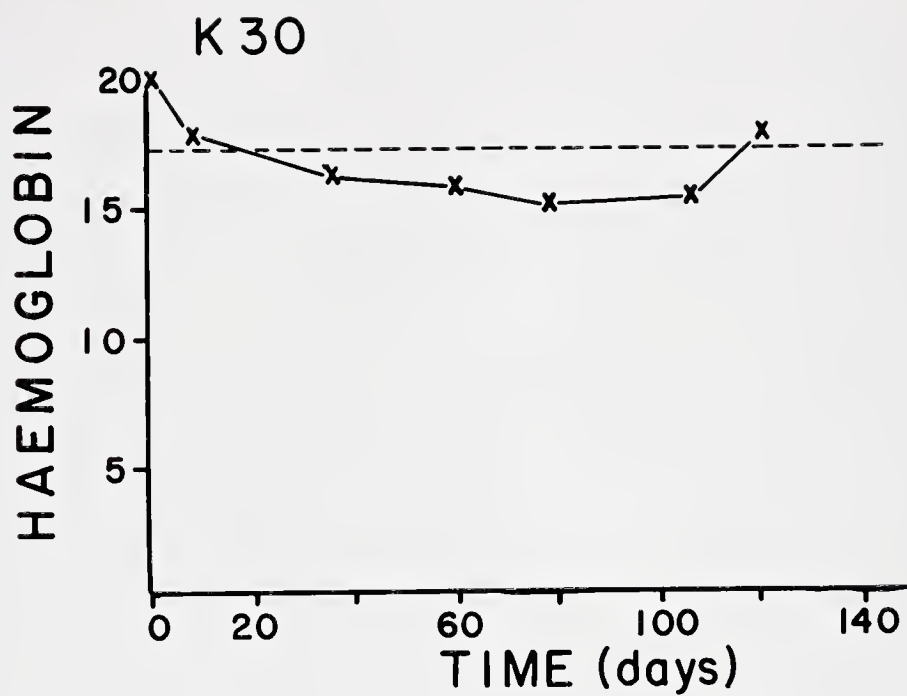


Fig. 106

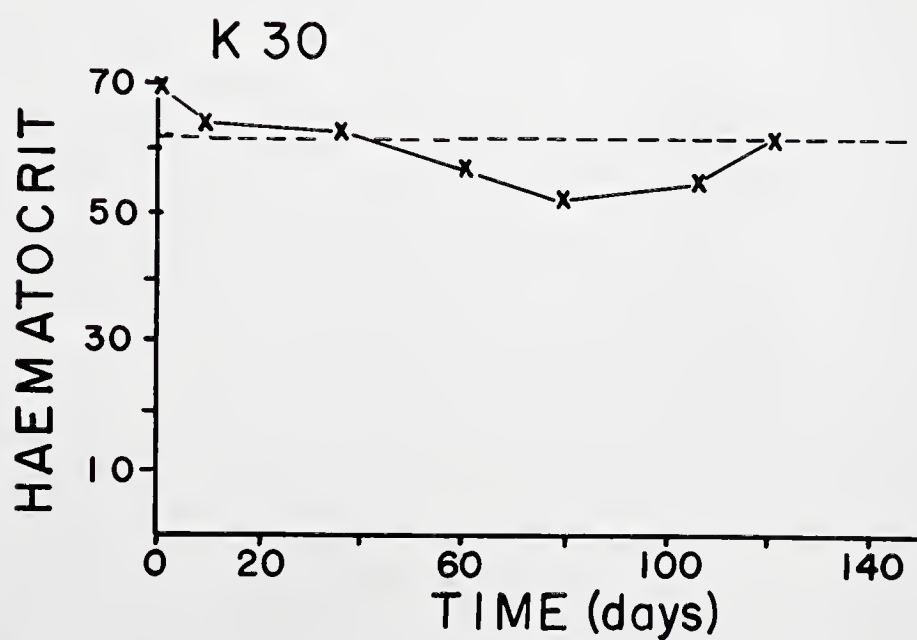


Fig. 107

DOG K-31

Hematological and biochemical investigation on the 15th of December, 1959, revealed only one abnormality of liver function, a raised Cephalin Cholesterol flocculation reading. Clinically the animal was alert, fit and healthy. Oral feeding with carbon tetrachloride in gelatin capsules commenced on the 16th of December, 1959. Initially the animal was given 1.5 ml. carbon tetrachloride every second to third day. The dose was gradually increased until at the time of laparotomy 112 days after the start of the experiment, 10 ml. carbon tetrachloride was being administered on alternate days. The dose of carbon tetrachloride was regulated by the level of SGOT. The aim was to keep the SGOT level below 100 units so that gross necrosis would be prevented. On two occasions, however, the level rose to between 300 and 400 units, but the dog remained clinically well and increased its weight from 20 kg. at the start of the experiment to 23 kg. at the time of laparotomy some 112 days later, during which period 280.75 ml. carbon tetrachloride were administered orally.

FINDINGS:

The liver appeared slightly smaller than would be expected in a dog of this weight, and was a pale brown in color. No nodularity was noted but the consistency of the organ was firm to rubbery. The spleen appeared normal and there was no evidence of a portal systemic collateral circulation or ascites. No other abnormality was noted.

The portal pressure measured 205 mm. saline.

HISTOPATHOLOGY

In this specimen there is a generalized centrolobular necrosis associated with a diffuse spidery increase in connective tissue which was evident as thin slivers or thickened sheets of collagen containing numerous young fibroblasts and in places an infiltration with inflammatory cells, mainly monocytic in type. This combination of degeneration, necrosis and fibrosis breaks up the normal lobular hepatic architecture rendering it rather fragmentary. Both Hematoxylin and Eosin and Reticulum staining techniques demonstrate this obscuration of the normal pattern. The latter stain in particular demonstrated the increase in reticulum in the centrolobular and portal areas, with septate increases in reticulum connecting centrolobular and portal areas, frequently making these areas the centers of star-shaped increase in reticulum staining material. Reticulum cells are the progenitors of the connective

tissue elements, and it is conceivable that an increase in reticulum may be followed by the laying down of connective tissue.

It is difficult, with the staining techniques used, to differentiate between an "active" increase in connective tissue, which is an absolute increase, and a "passive" increase which is due to a relative increase when collapsed areas of cells and tissue framework are compressed to produce thickened bands. However, where the fibroblasts are abundant, stain deeply, have large deeply stained nuclei and lie in an eosinophilic collagen-like material, the likelihood is that the increase is active.

In some areas the centrolobular fields are spared, and the increase in fibrosis is mainly portal.

Beginning regeneration is noted in numerous areas, mainly at the periphery of lobules, where the liver plates are seen to be more than one cell thick. Some of these areas have produced "passive" septums at their circumferences, thereby assuming the appearance of nodules.

The evidence of cirrhosis in this specimen is not overwhelming, but sufficient criteria are present to warrant the description of early diffuse portal or septal cirrhosis. The process is not sufficiently advanced to cause widespread collapse of the central veins or portal tributaries. This may account for the absence of ascites and the moderate rise in portal pressure. (See Figs. 108-111)

BIOCHEMISTRY

The results of the biochemical investigation in this case did not demonstrate any real tendency towards hepatic dysfunction, apart from an increase in BSP retention. This parameter was noted to rise after the first month, remain elevated for some 50 days, descend to a lower but still abnormal level then rise again during the last three weeks of the experiment. This correlates to some extent with changes in SGOT levels. The Serum Alkaline Phosphatase fluctuated within the limits of normality, as did the Serum Cholesterol and Serum Bilirubin. The Cephalin Cholesterol flocculation rose towards the end of the experiment from 3 to 4 plus units.

The BUN rose initially then decreased, remaining all the while within the limits of normality.

The Serum Protein changes were similar to those described in the reports on the preceding dogs, and the Total Serum Protein level diminished slightly during the first 70 days of the experiment then rose to the elevated level of 10.3 gm.% by the 100th day but subsided once again to the pre-experimental level by the termination of the project. The Serum Albumin level made a gradual fluctuating but slight descent.

The Serum Globulin after fluctuating around the average normal level for the first $2\frac{1}{2}$ months made a steep ascent to a level in excess of 6 gm.%. This coincided with, and obviously accounted for the rise in the total serum proteins which occurred simultaneously. The highest level of SGOT was observed during this period as well. The Albumin-Globulin ratio also demonstrated a fluctuant tendency with a reversal to 0.6 which coincided with the high SGOT and Total Serum Protein levels. There appears to be a definite relationship in this instance between hepatocellular damage as indicated by SGOT level and protein metabolism. (See Figs. 112-122)

HEMATOLOGY

The changes in the hemoglobin and hematocrit in this animal were remarkably similar to those seen in Dog K-30. Both decreased slightly over the first half of the experimental period then gradually increased, remaining at all times within the limits of normality. (See Figs. 123, 124)



Fig. 108



Fig. 109

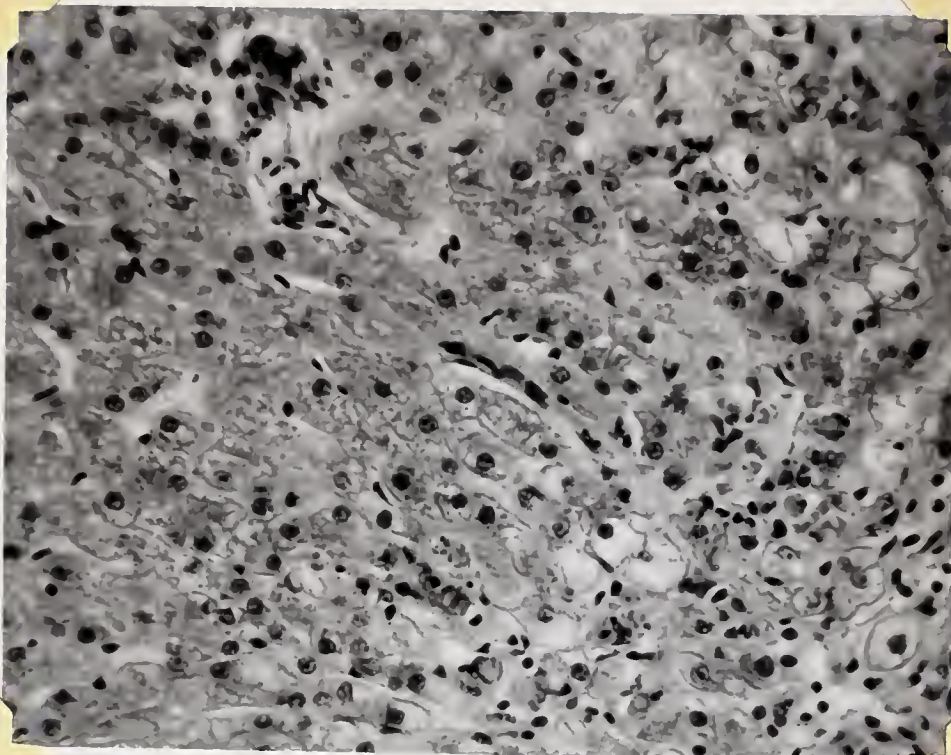


Fig. 110

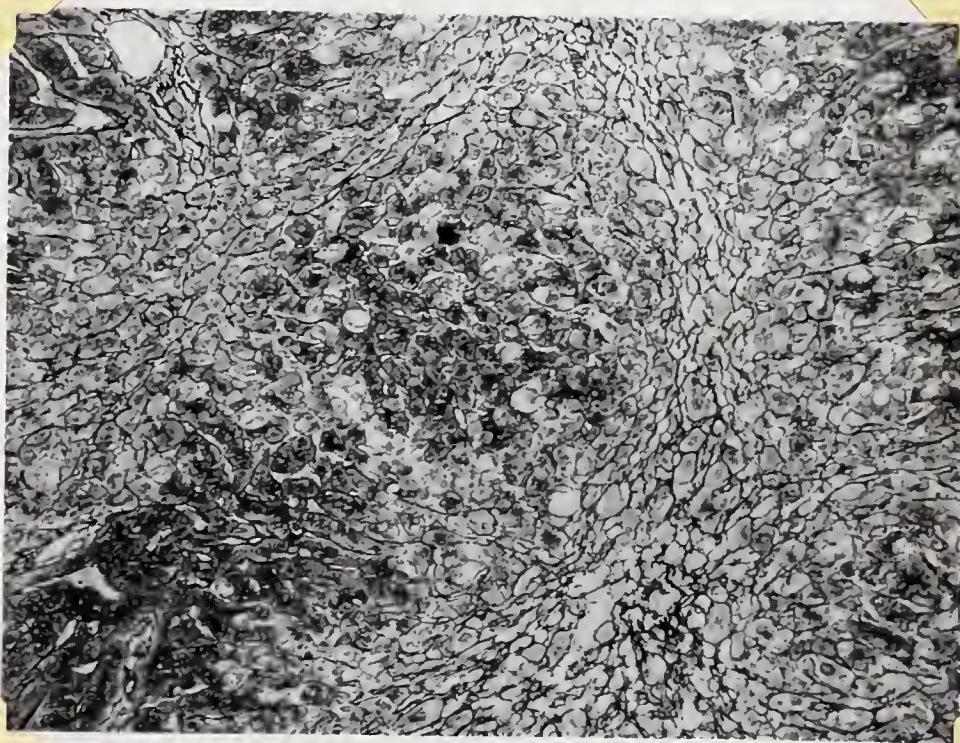


Fig. 111

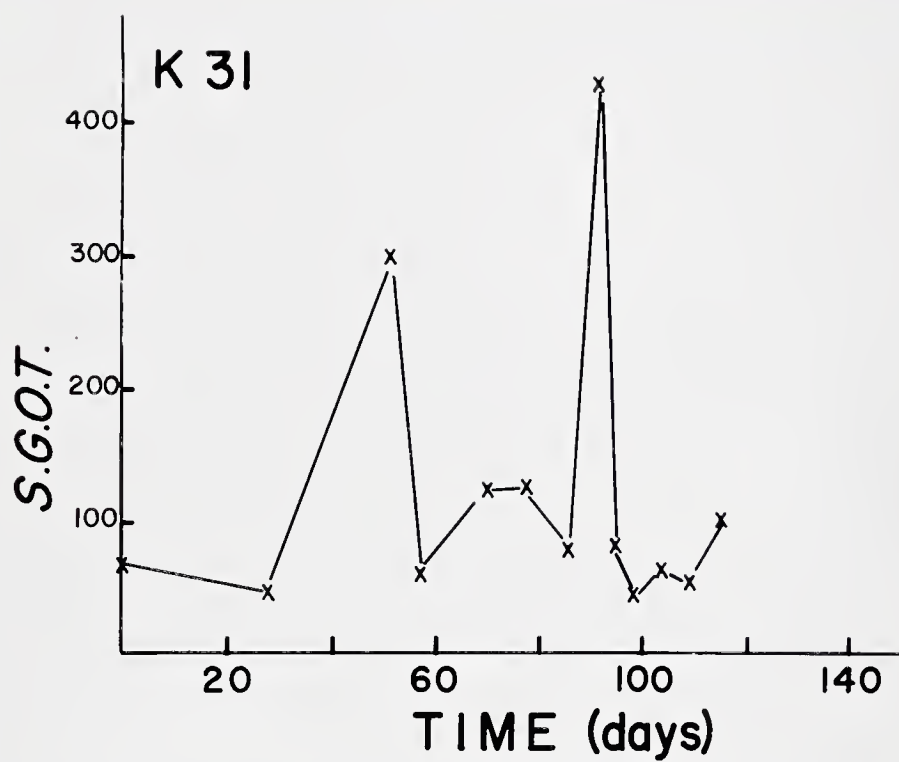


Fig. 112

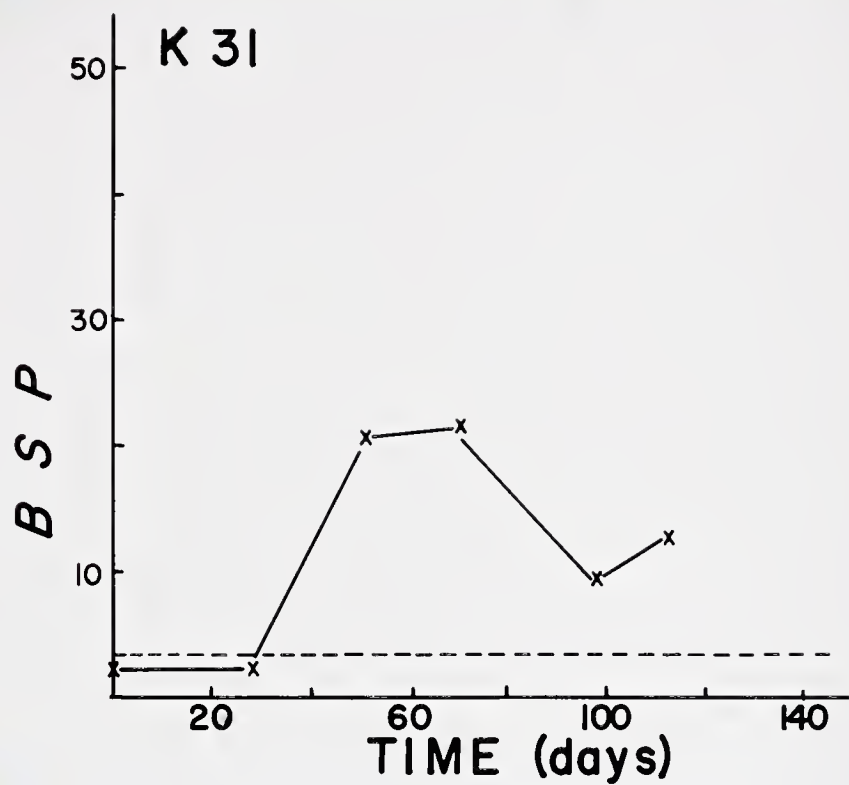


Fig. 113

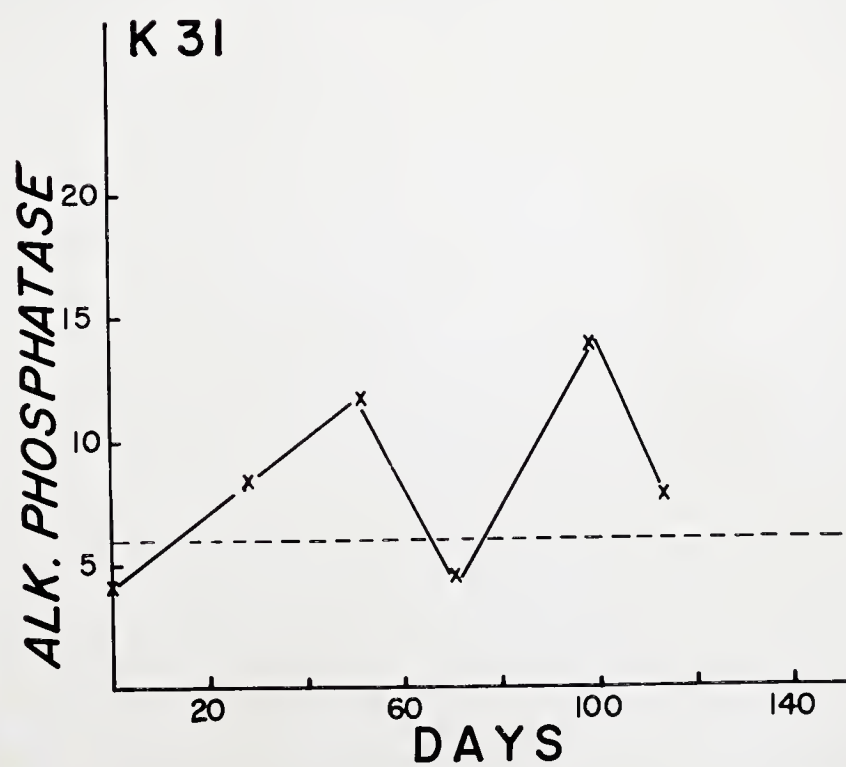


Fig. 114

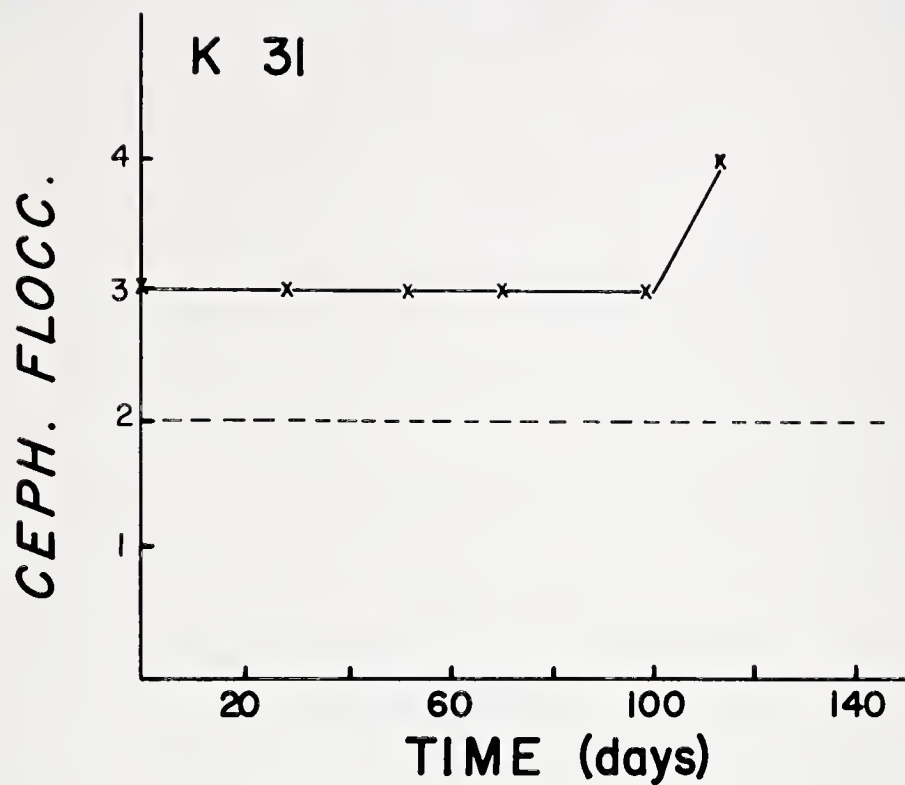


Fig. 115

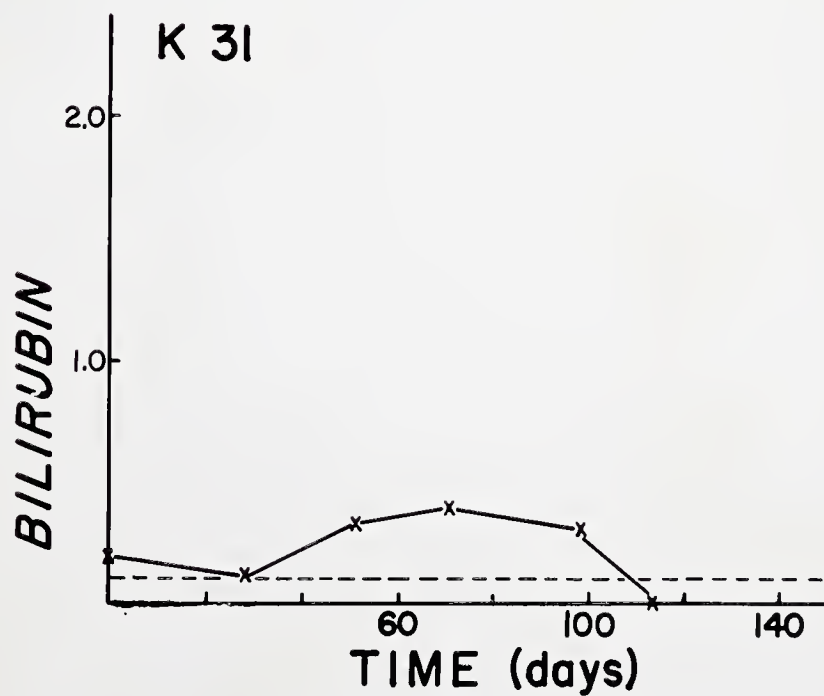


Fig. 116

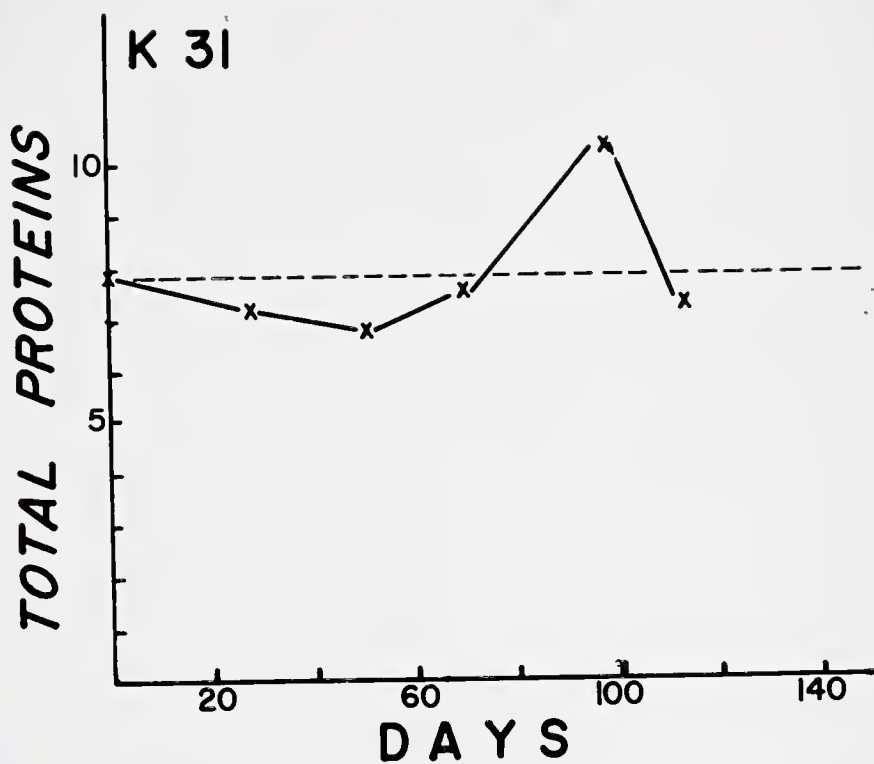


Fig. 117

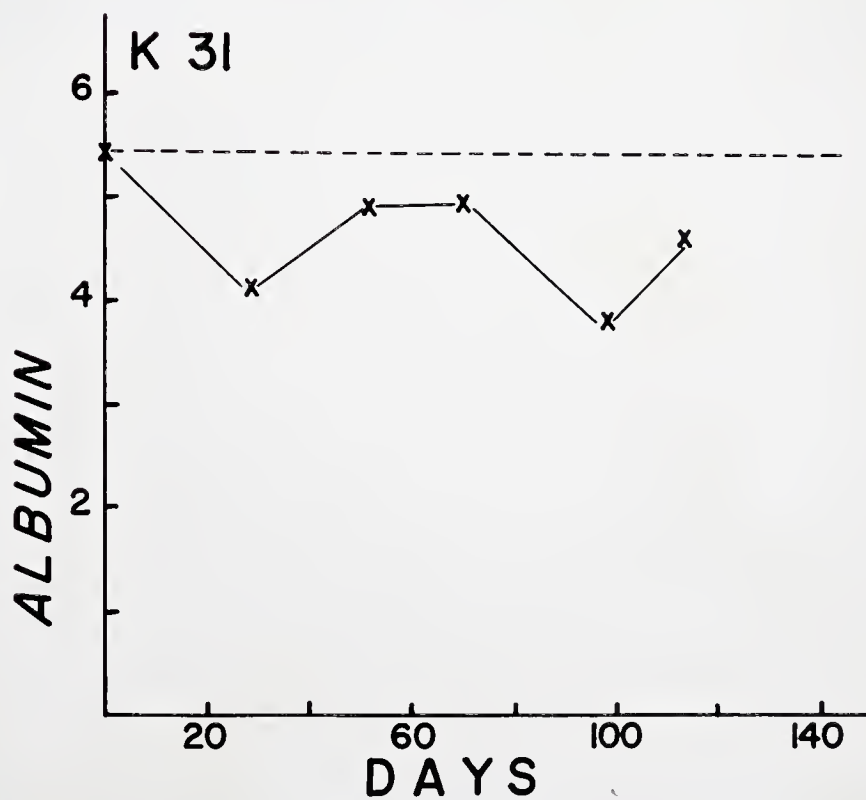


Fig. 118

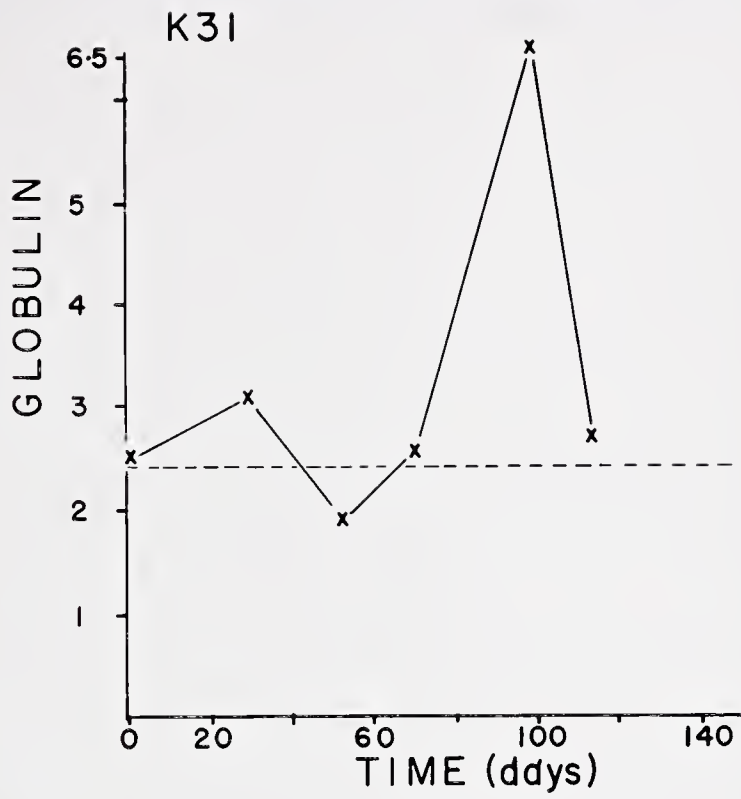


Fig. 119

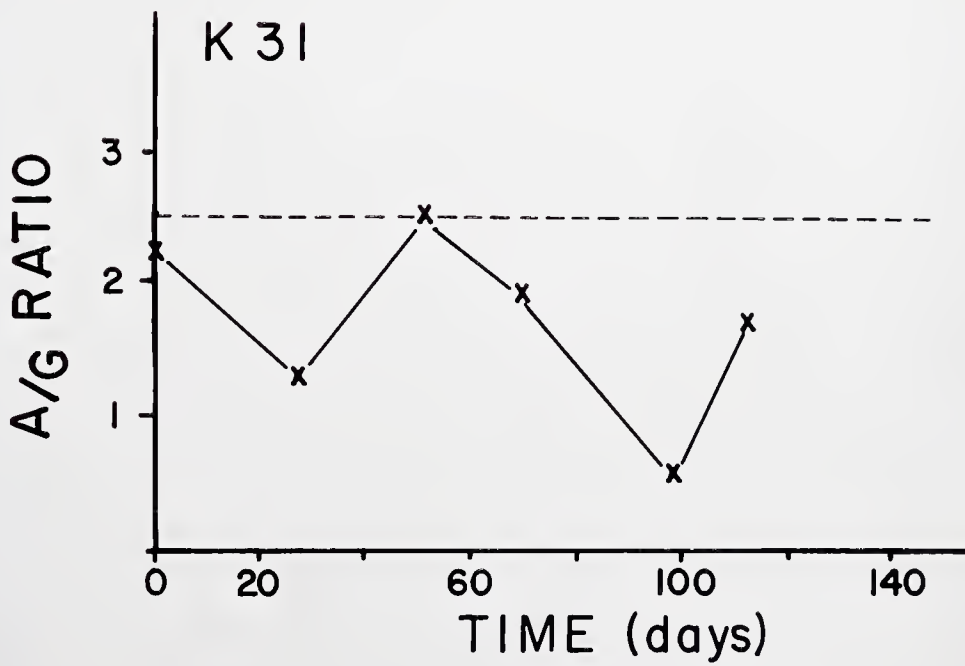


Fig. 120

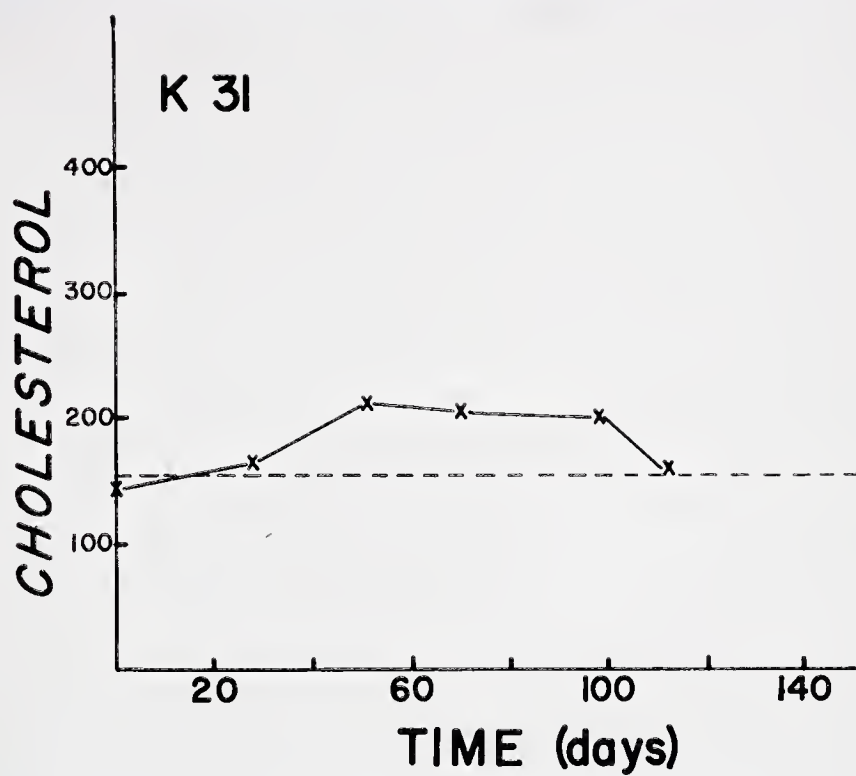


Fig. 121

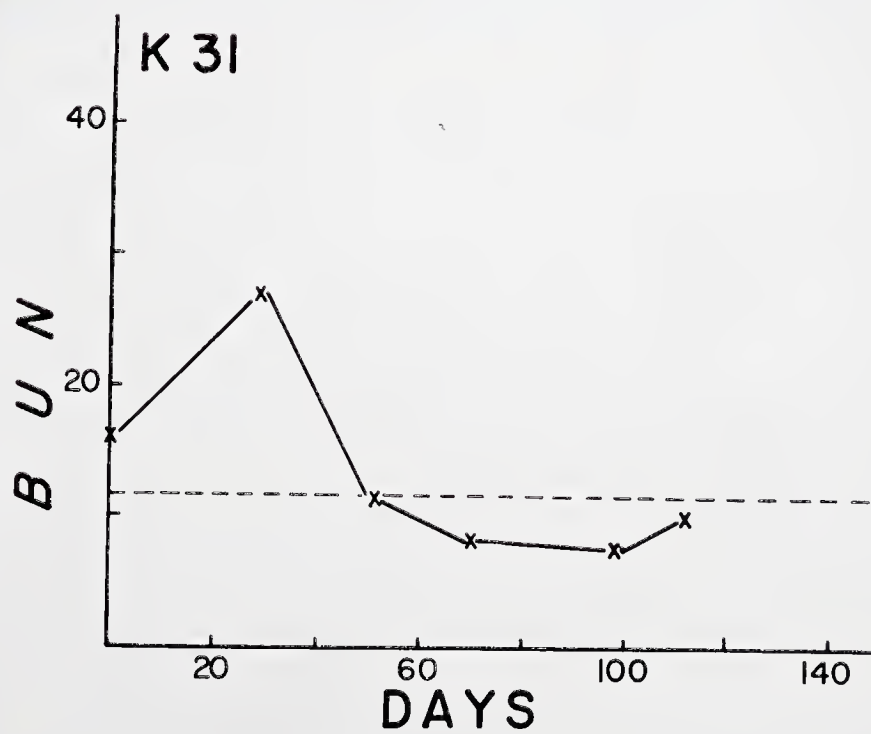


Fig. 122

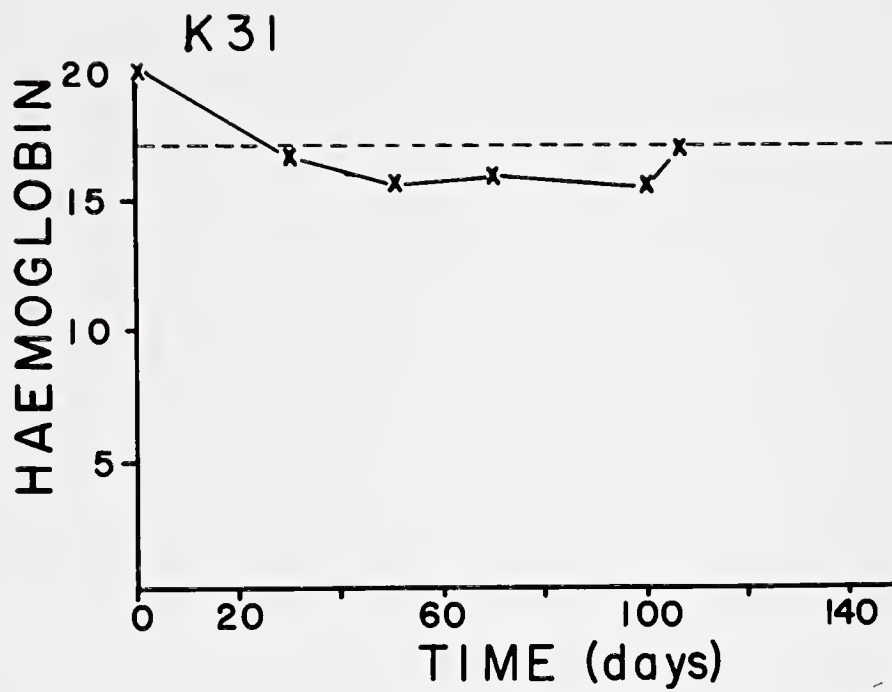


Fig. 123

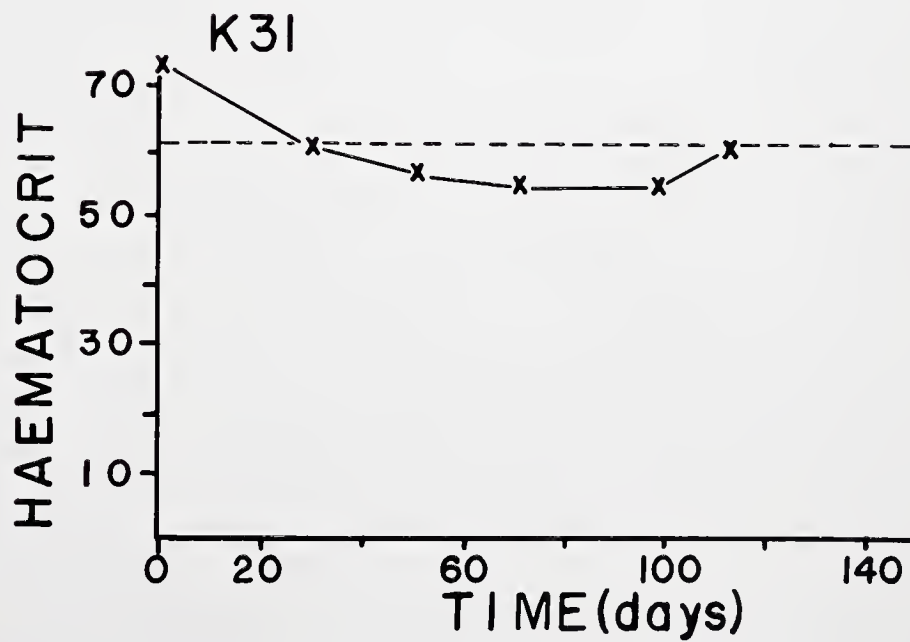


Fig. 124

DOG K-32

Hematological and biochemical investigation on the 18th of December, 1959, revealed no abnormality of liver function. Clinically the animal was alert, fit and healthy, weighing 22.5 kg. Oral feeding with carbon tetrachloride in gelatin capsules commenced on the 27th of December, 1959. Initially the animal was given 1.5 ml. carbon tetrachloride every second to third day. This was gradually increased until at the time of demise, 87 days after the start of the experiment the dog was receiving 5 ml. carbon tetrachloride on alternate days. Two weeks after the onset of the experiment the dog became ill and was noted to vomit intermittently. There had also been a weight loss of 3 kg. It was assumed that a too rapid and severe hepatic necrosis was being produced, so the carbon tetrachloride was discontinued for two weeks. 250 mg. of terramycin were given intramuscularly for the first three days of the illness. After the two week period, the dog regained his health and carbon tetrachloride administration was again instituted. An initial dose of 0.75 ml. was given followed by 1.5 ml. two days later, after which the

dosage was gradually increased again. Death occurred on the 16th of March, 1960, the day after 15 ml. carbon tetrachloride had been introduced into the dog's stomach by means of a nasogastric tube. This procedure was performed under general anaesthesia induced by administration of Nembutal intravenously. The dosage of Nembutal used was 0.5 ml. per kilogram body weight. The dog never regained consciousness and succumbed the day after the carbon tetrachloride intubation. The cause of death was presumed to be due to Barbiturate poisoning, in view of the diseased liver which was unable to detoxify the Nembutal. Measures were taken to counteract this intoxication including the administration of Megimide and Picrotoxin intravenously, as well as an intravenous transfusion of 5% Dextrose in water, but to no avail.

During the period of the experiment, 87 days in this instance, 140.25 ml. carbon tetrachloride were administered to the animal. The dog's weight decreased from 22.5 kg. initially to 16.5 kg. at the time of induction of the fatal anaesthetic. The dog appeared clinically well during the period prior to death but had exhibited indifferent health during the total period of the experiment.

FINDINGS:

A post mortem examination performed shortly after the animal's death revealed the following findings.

The liver was small, mottled, pale brown in color and finely nodular in appearance. It weighed 384 gm. and was of a rubbery consistence. On section it was coarse. There was no evidence of an increased portal systemic collateral circulation, but this was a difficult aspect to ascertain at autopsy. The spleen appeared normal and ascites was not present. Both lungs were atelectatic.

HISTOPATHOLOGY

This specimen exhibits a generalized centrolobular necrosis with widespread bridging of the necrotic areas. This produces the diffuse appearance of areas of healthy cells lying periportally, surrounded by cells demonstrating all the changes of degeneration up to and including necrosis.

Early disorganization of the hepatic architecture is evidenced by the following findings. (1) Centrolobular areas are the seat of degenerative changes. (2) Positions of the portal tracts and central veins have been altered so that their distribution is no longer diffusely regular. Central veins may be found lying next to each other with only narrow intervening areas of necrotic and collapsed cells, or they may lie in fairly close proximity to portal

tracts for the same reason. The reticulum stained specimens demonstrate these aspects, showing also a slight parenchymal increase in reticulum. The central veins and portal tracts are seen to be patent, with very little evidence of collapse. (3) There is a moderate increase in the number of inflammatory cells present, mainly monocytic in type. These cells are aggregated mainly in the degenerative areas. (4) In some areas sheets of fairly healthy looking connective tissue are present, usually in relationship to portal tracts.

There is minimal evidence of hepatocellular regeneration, seen as beginning regeneration in the peripheral regions of the lobules. It is recognized as cell layers two or more cells in thickness.

It is possible that the death of this animal following gastric intubation with carbon tetrachloride under Nembutal anesthesia, has resulted in a greater degree of necrosis due to an agonal centrolobular necrosis associated with acute cardiac failure and anoxia.

The findings in this case do not appear to be inconsistent with the short period of carbon tetrachloride intoxication to which the animal was submitted. (87 days).

Although one cannot label this specimen as cirrhosis, the appearances are indicative of a transformation into a diffuse form of cirrhosis. It is suggested

that the relatively unaffected areas around the portal tracts regenerate and hypertrophy to produce regenerative nodules, while the collapsed central areas are compressed into the so-called "passive" septums. The picture would be completed by a diffuse increase in connective tissue and bile duct proliferation, of which there is very little evidence in this specimen.

In the light of the findings, this specimen is interpreted as a diffuse hepatic degeneration with early evidence of cirrhotic transformation. (See Figs. 125-129).

BIOCHEMISTRY

The SGOT level was maintained between 50 and 150 units except for two occasions when it rose to 271 and 192 units respectively. The high level of Serum Alkaline Phosphatase and BSP retention was associated with the first elevation of the SGOT. The BSP retention rose gradually throughout the period of the experiment reaching an abnormal level in the first two weeks. The Serum Alkaline Phosphatase rose to the high level of 42.6 K.A. units, remained elevated, then descended to a normal level by the 6th day.

The Serum Bilirubin demonstrated a gradual increase throughout the period of the experiment.

The Cephalin Cholesterol flocculation was at the 3 plus level to start with, rose to 4 plus at the time of the first SGOT increase, then returned to the 3 plus level.

The Serum Cholesterol fluctuated slightly within the limits of normality.

The BUN demonstrated a gradual decrease throughout the period of the experiment, but was at no

time abnormally low.

The changes in the Serum Proteins were similar to those seen in most of the other dogs. The Total Serum Protein level decreased by 2 gm.%. The Serum Albumin level though it fluctuated, finally decreased by some 2.5 g.m% while the Serum Globulin level demonstrated a gradual increase of 1 gm.%. The Albumin-Globulin ratio decreased, at one stage being reversed. (See Figs. 130 - 140).

HEMATOLOGY

Both the Hemoglobin and Hematocrit levels decreased gradually throughout the period of the experiment. (See Figs. 141, 142).

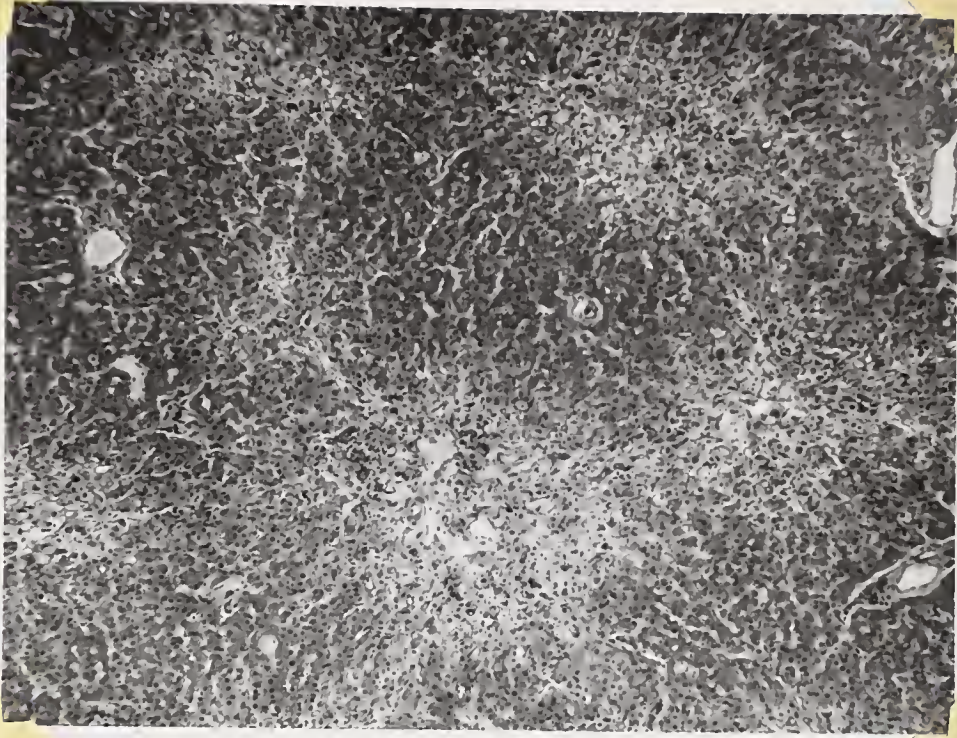


Fig. 125



Fig. 126

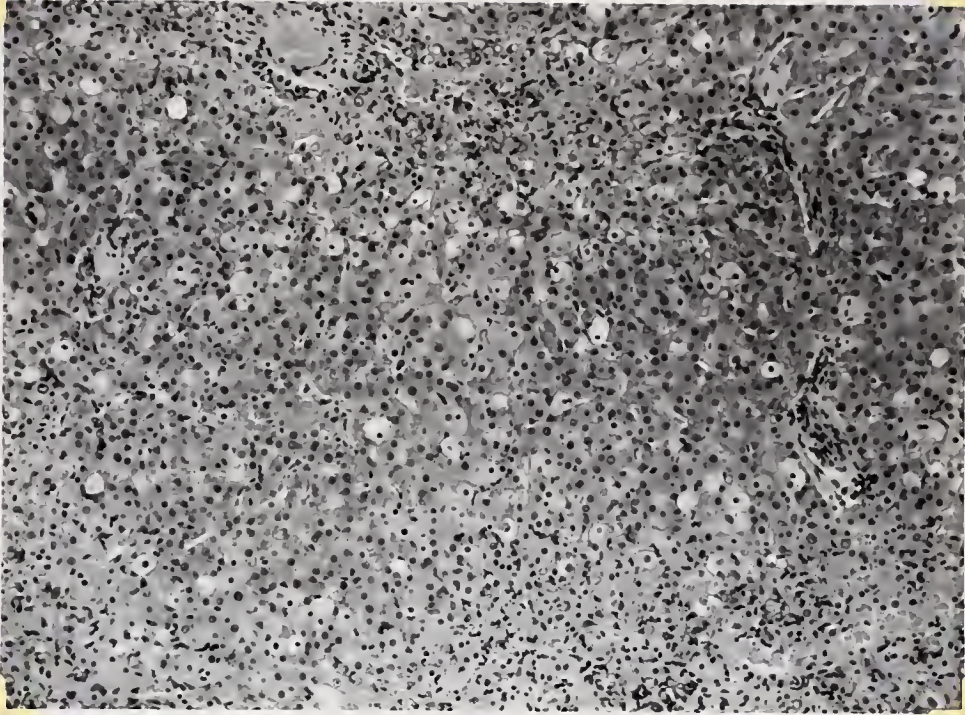


Fig. 127

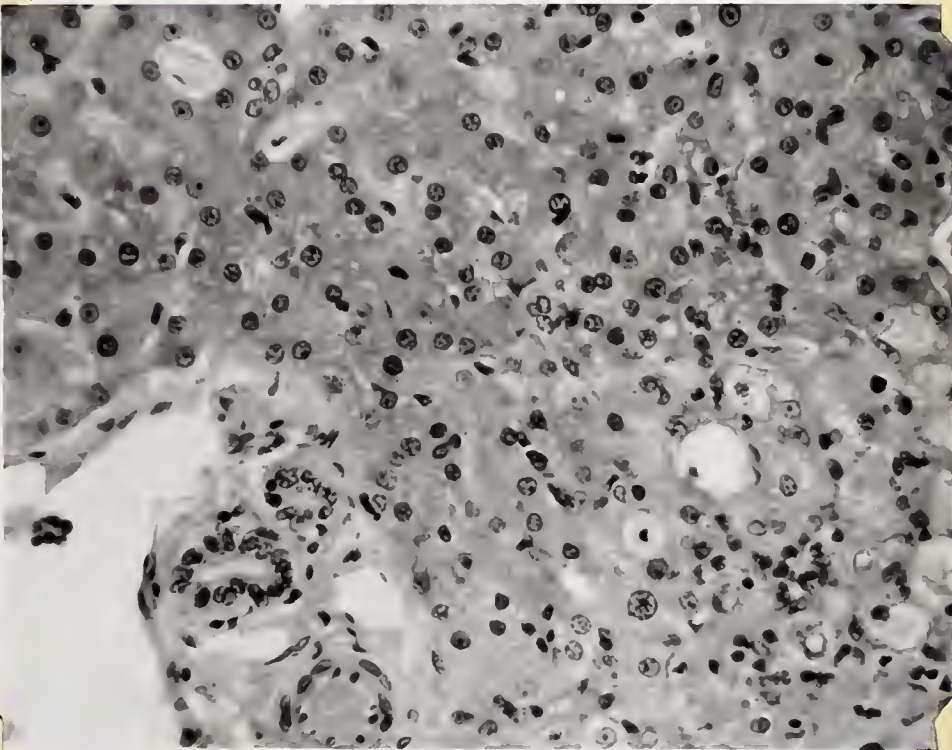


Fig. 12 8

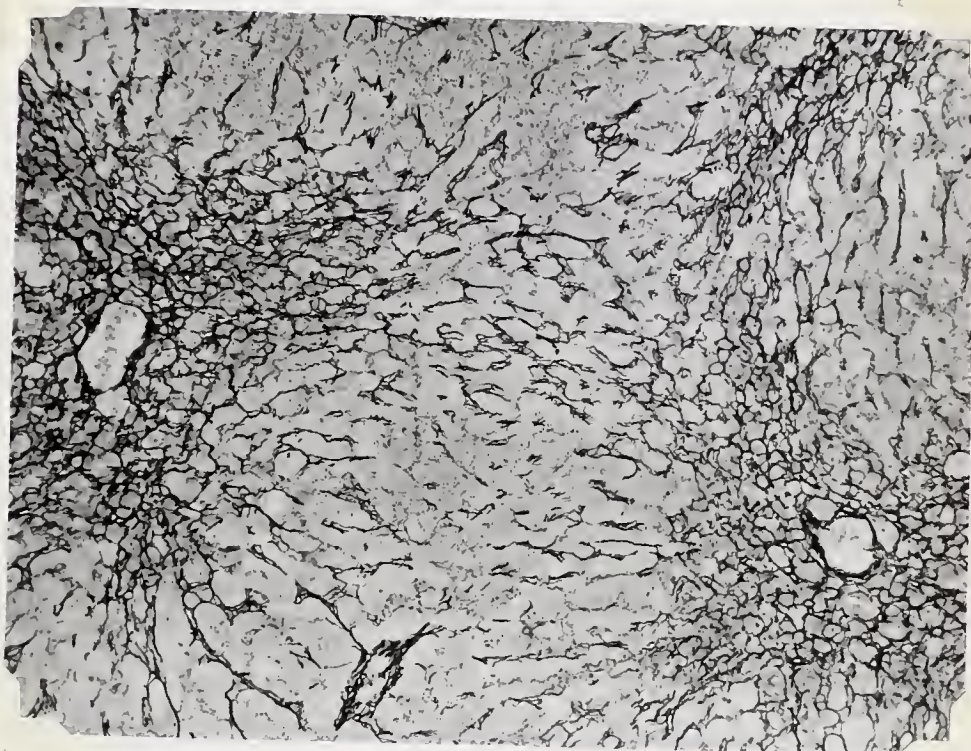


Fig. 129

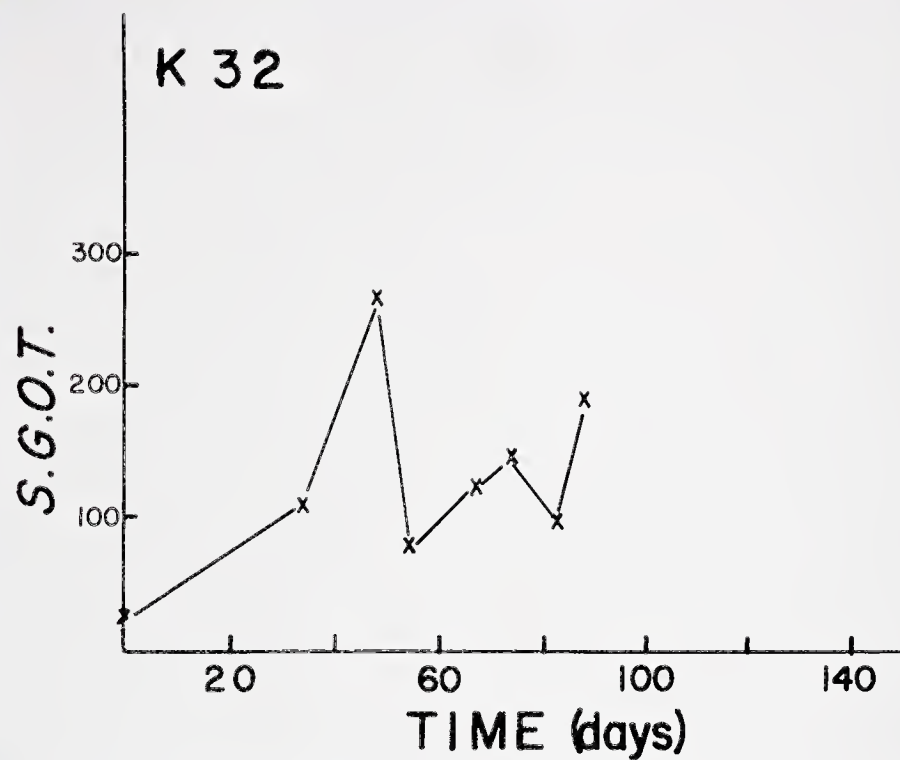


Fig. 130

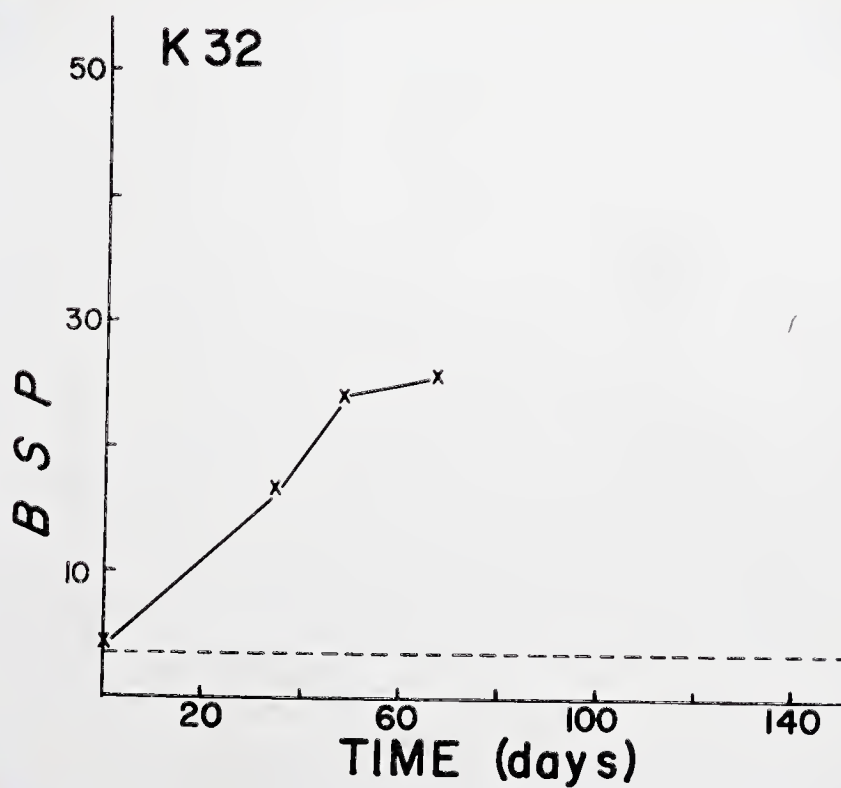


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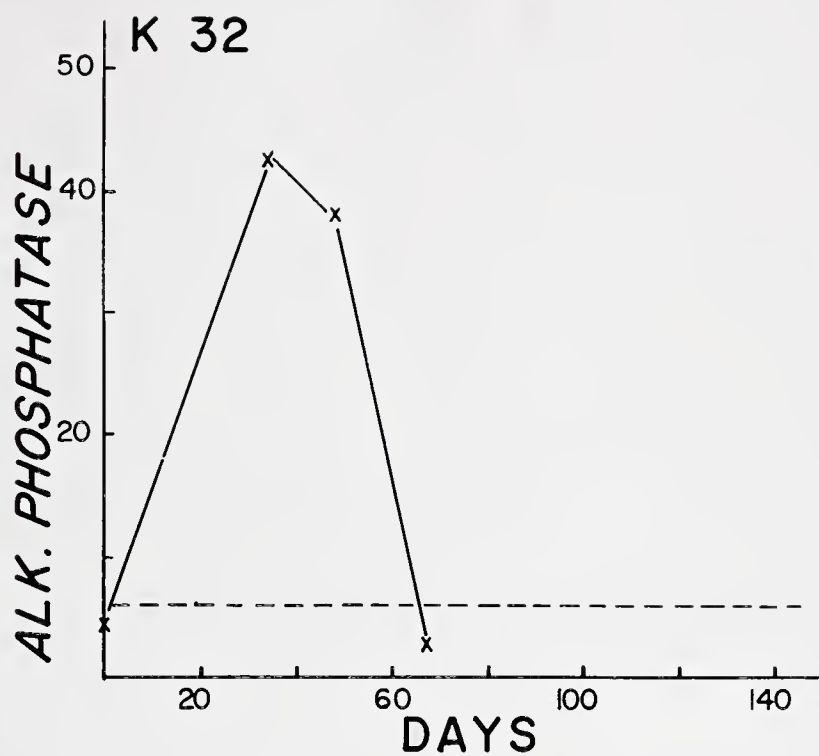


Fig. 132

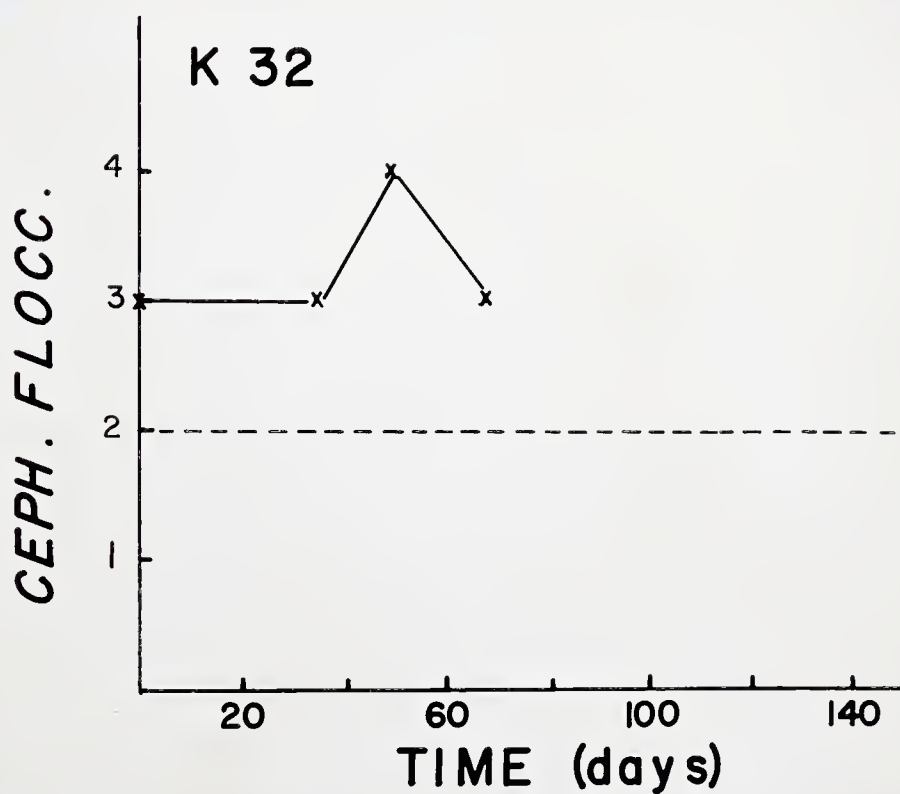


Fig. 133

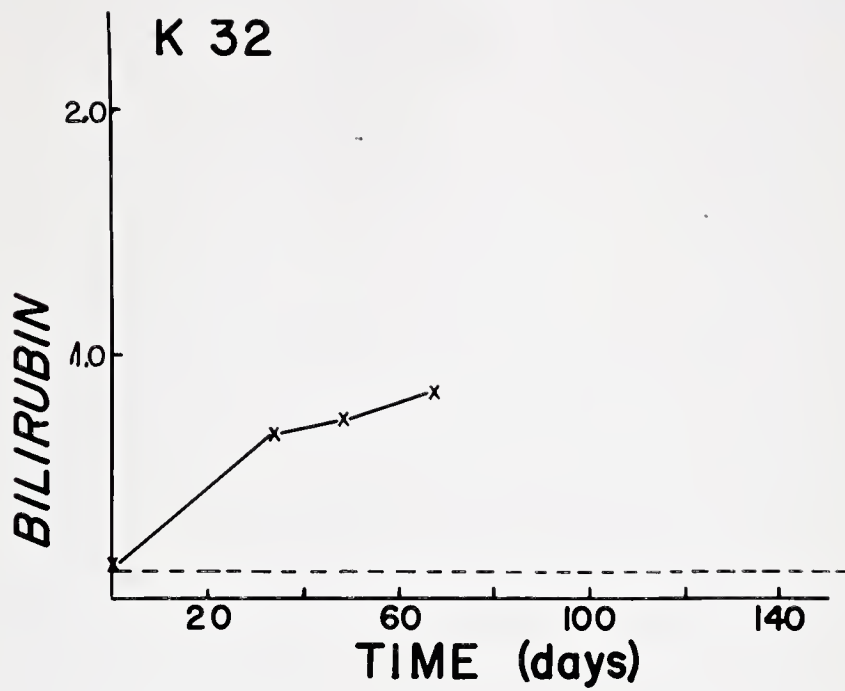


Fig. 134

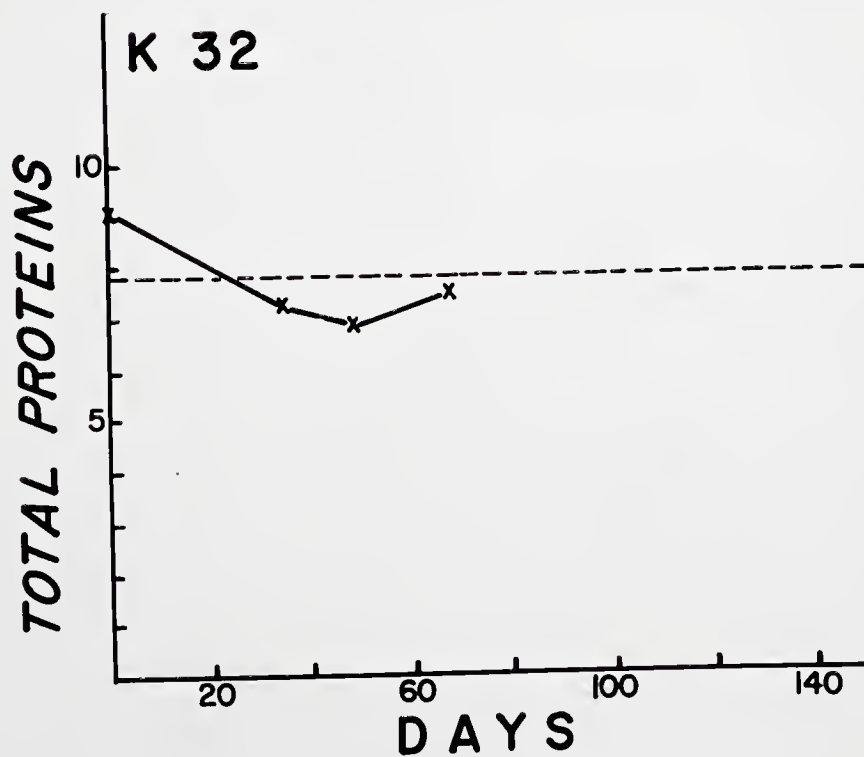


Fig. 135

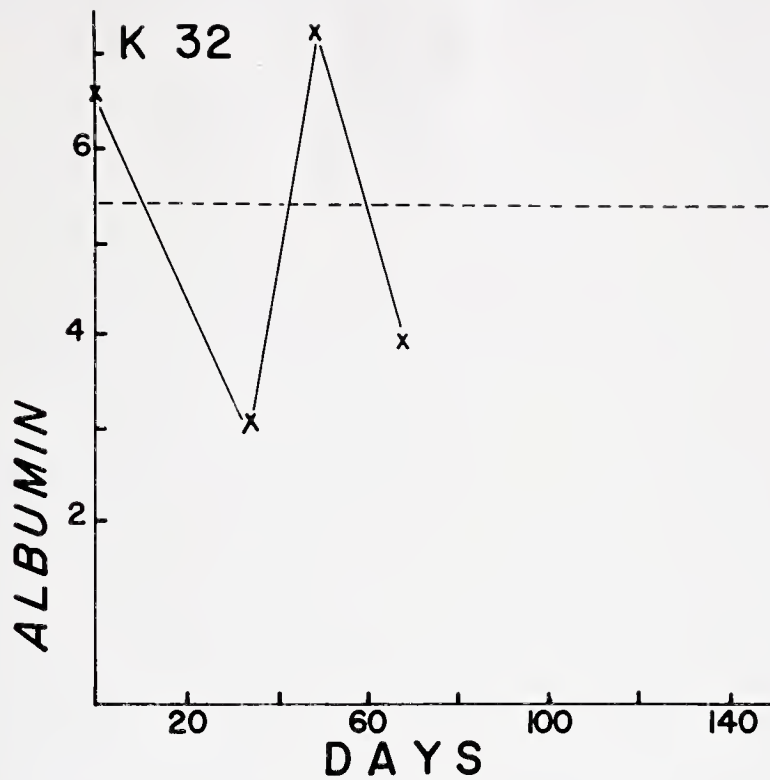


Fig. 136

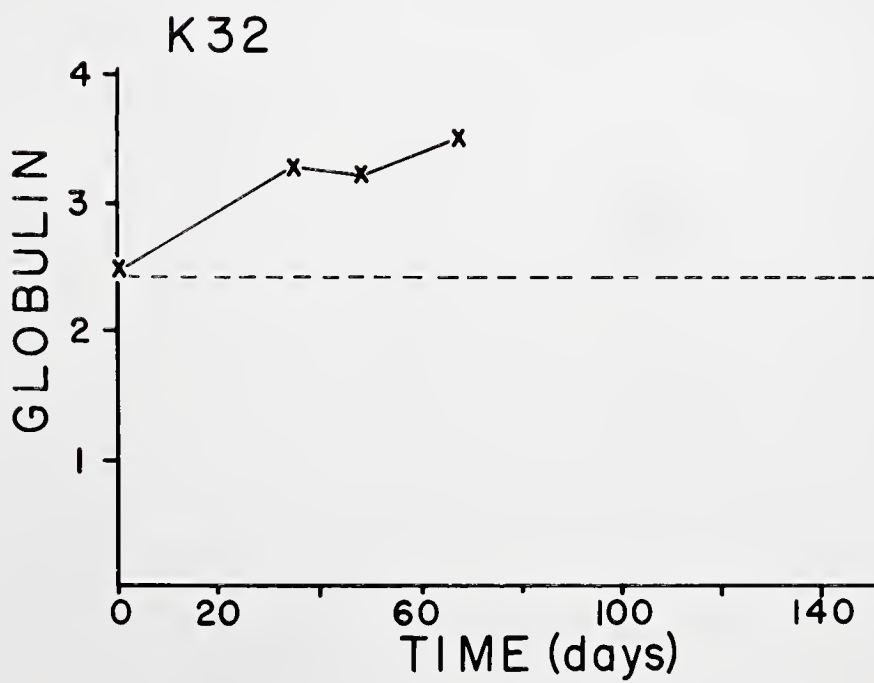


Fig. 137

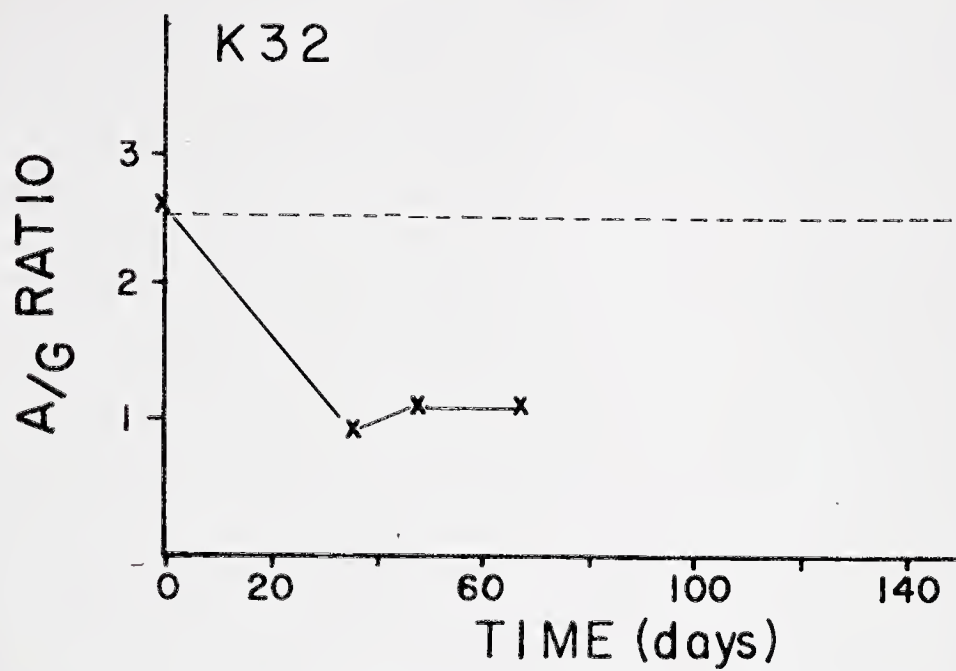


Fig. 138

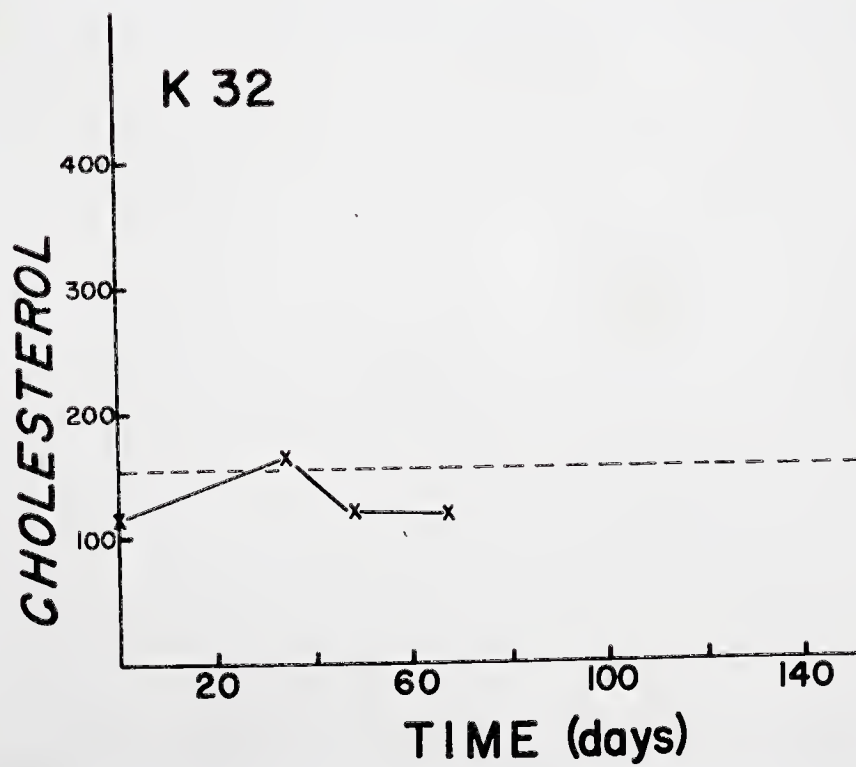


Fig. 139

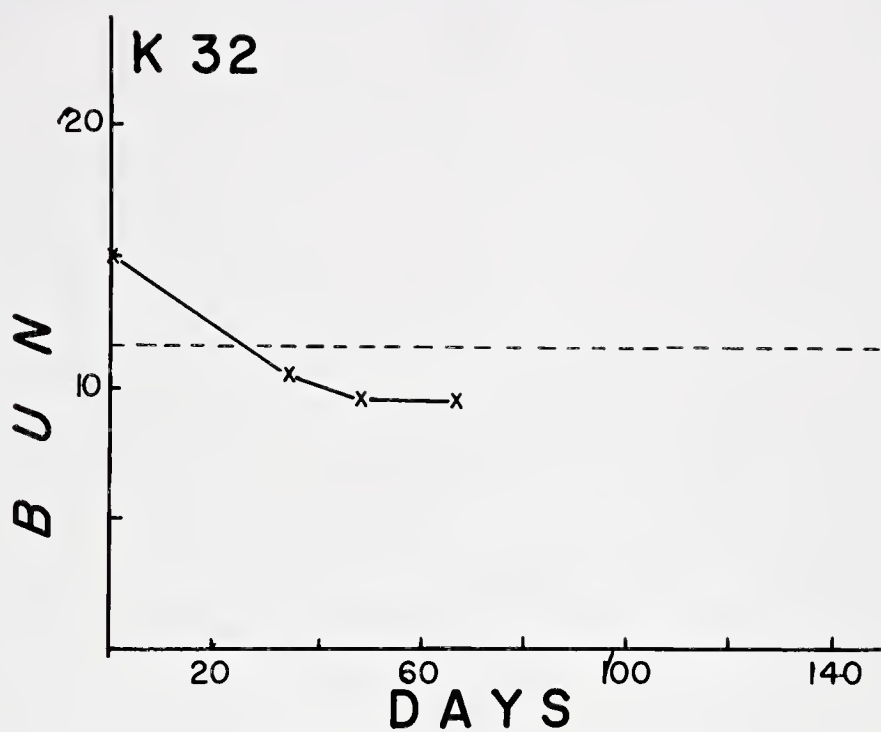


Fig. 140

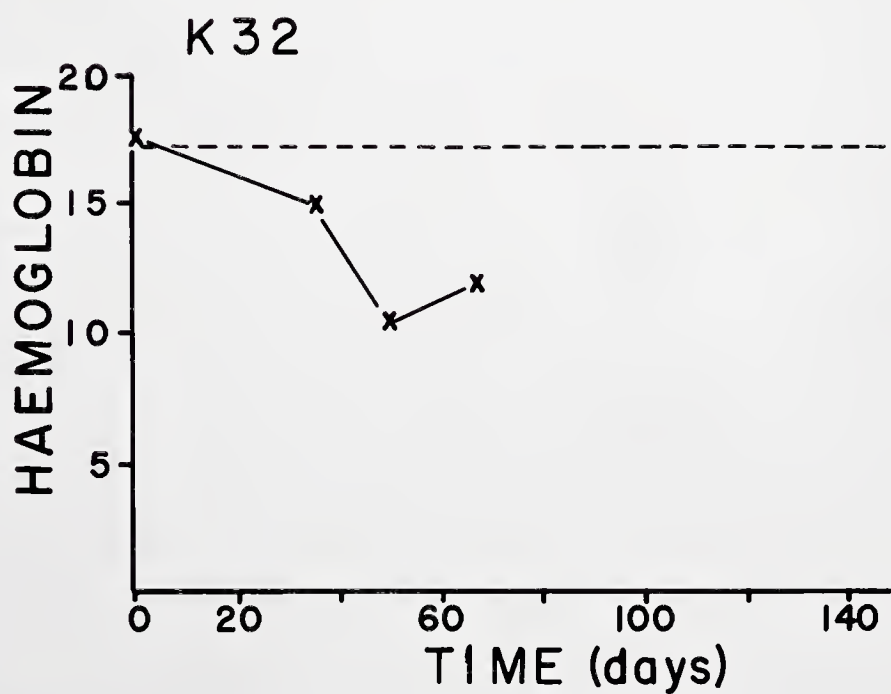


Fig. 141

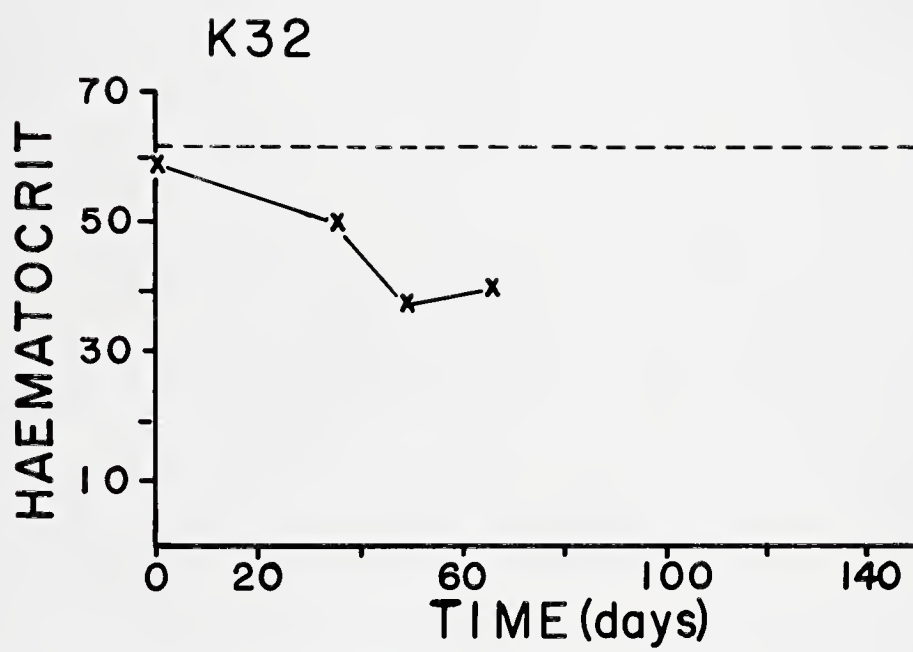


Fig. 142

DOG K-33

Hematological and biochemical investigation on the 29th of December, 1959, revealed no abnormality of liver function. Clinically the animal was alert, fit and healthy. Oral feeding with carbon tetrachloride in gelatin capsules commenced on the same date following the extraction of blood for testing. Initially the animal was given 1.5 ml. carbon tetrachloride every second to third day. The doses gradually increased until at the time of laparotomy on the 8th of April, 1960, 102 days after the start of the experiment, 10 ml. carbon tetrachloride were being administered on alternate days.

An attempt was made to regulate the dose of carbon tetrachloride by intermittent SGOT estimations, but in this animal it proved exceedingly difficult as reference to the graph on SGOT level clearly demonstrates.

The dog remained clinically healthy, even in the presence of markedly raised SGOT levels. The animal's weight decreased by 1.25 kg. from the initial level of 17.75 kg. to a preoperative level of 16.5 kg. During the 102 days in the experiment, the dog received 243.25 ml. carbon tetrachloride via the oral route.

FINDINGS

Liver, which was a yellowish brown in color, exhibited an obviously nodular surface. The nodules varied in size from 1 to 3 mm. The organ appeared to be slightly enlarged. On palpation, the consistency was very firm to rubbery, a characteristic which was confirmed when the organ was incised during the biopsy.

There was no evidence of ascites or increased portal systemic collateral circulation. The spleen appeared normal. No other abnormality was noted. The portal pressure measured 180 mm. of saline.

HISTOPATHOLOGY

In this specimen, both the Hematoxylin and Eosin and Reticulum staining techniques have clearly demonstrated the disorganization of the lobular architecture which is barely recognizable. The outstanding features of this specimen are, (1) Disorganization of the hepatic architecture, (2) Diffuse increase in connective tissue, (3) Marked increase in bile duct proliferation, (4) Marked increase in inflammatory cells, (5) Diffuse nodular regeneration.

The parenchyma is diffusely subdivided by connective tissue septums. In some areas these are probably due to collapse following hepatocellular necrosis, while in others the appearance is that of

healthily staining fibroblasts encased in young collagen.

All stages of degeneration up to and including necrosis are present. There is a moderate proliferation of Kupffer cells and in the fibrous septums, bile and blood pigment bearing macrophages are noted.

The septums subdivide the parenchyma into small islands while the portal tracts appear to be the seat of a moderately marked increase in connective tissue.

The vascular pattern of this specimen is not clearly discernible and the central veins appear to be largely collapsed.

The reticulum stain demonstrates quite well the formation of "passive" septums around the nodules, while the increase in reticulum staining material is noted to be diffuse in its pattern, with perhaps some increase in the portal areas.

This specimen would appear to demonstrate adequately the criteria associated with cirrhosis of the diffuse septal or portal variety, following the relatively short period of 102 days carbon tetrachloride intoxication. (See Figs. 142 - 145)

BIOCHEMISTRY

The SGOT level was most irregular during the course of this dog's experimental period. On three occasions it was elevated to exceedingly high levels, once over 350 units, while on two other occasions it was about 500 units. For the rest of the experimental period the SGOT level fluctuated between 50 and 150 units.

The BSP retention level was markedly raised at the time of the first SGOT elevation, descended simultaneously with the SGOT, then rose again at the same time that the second and third SGOT elevations occurred. From the time of the first reading following the administration of carbon tetrachloride, the BSP retention remained significantly raised.

The Serum Alkaline Phosphatase ascended early in the experiment and remained significantly elevated until just prior to laparotomy when it reverted to a normal level.

The Serum Bilirubin and Cephalin Cholesterol flocculation levels imitate the SGOT graph, remaining significantly raised until the termination of the experiment.

The Serum Cholesterol fluctuated within the limits of normality, the fluctuations corresponding to

the fluctuations of SGOT. Apart from its early elevation the BUN varied little from its normal pre-experimental level.

The Total Serum Proteins demonstrated a gradual decrease of approximately 1.5 gm. %. This correlated fairly closely with a decrease in Serum Albumin but the Serum Globulin, although it was raised on several occasions, usually descended to the pre-experimental level. The Albumin-Globulin ratio showed an inverse relationship to the Serum Globulin. On one occasion it was reversed. (See Figs. 146-156).

HEMATOLOGY

The Hemoglobin and Hematocrit estimations did not change significantly throughout the experiment. (See Figs. 157, 158).



Fig. 143

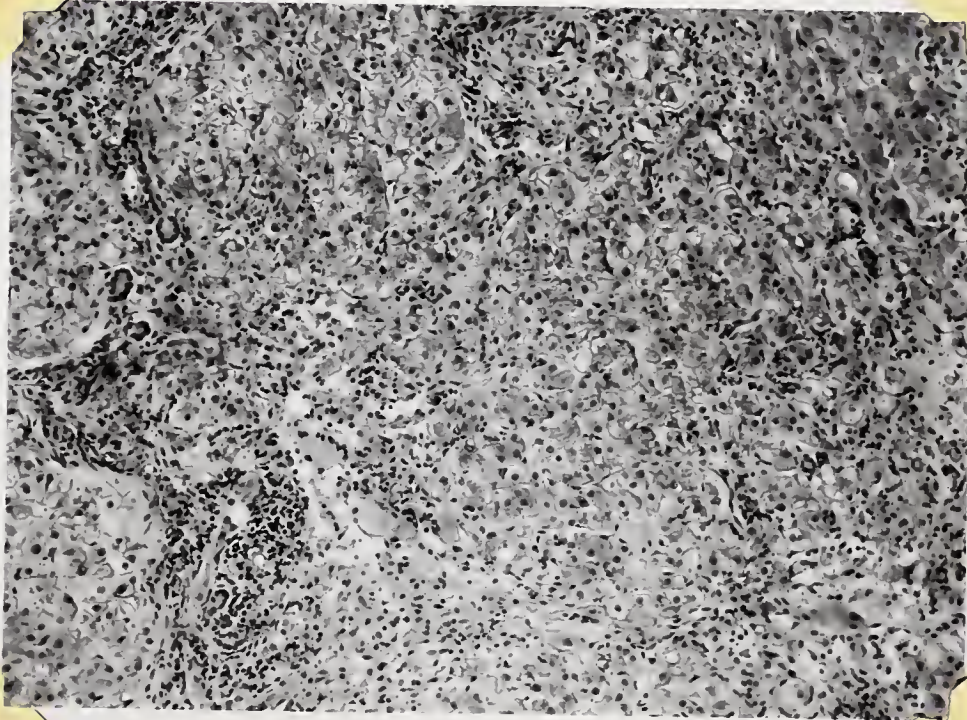


Fig. 144

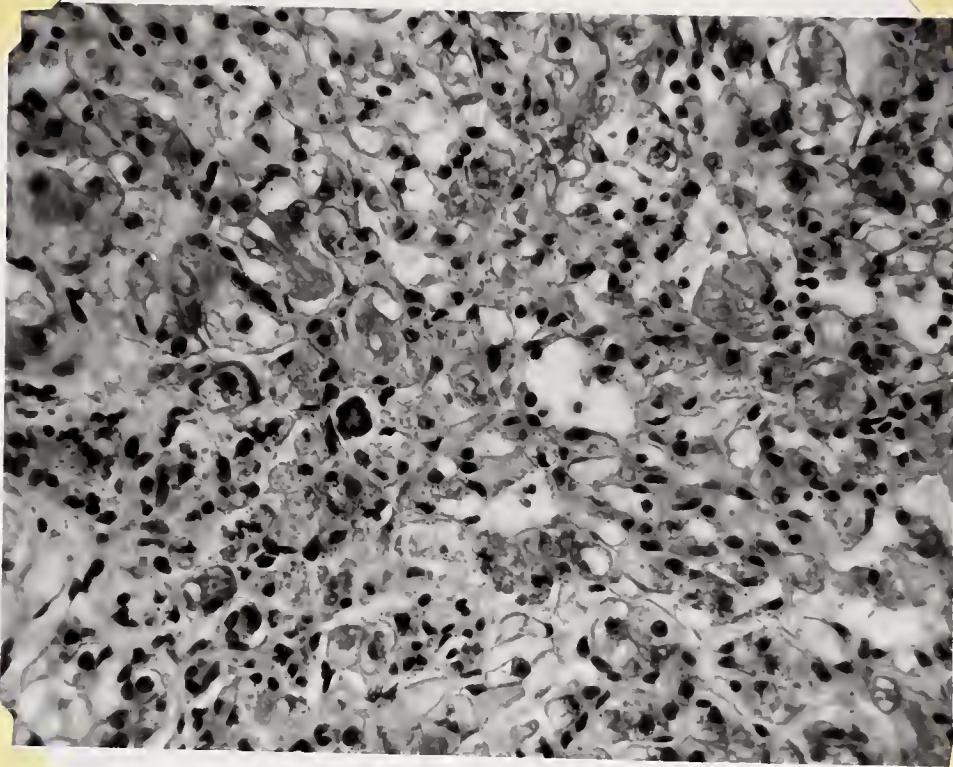


Fig. 145

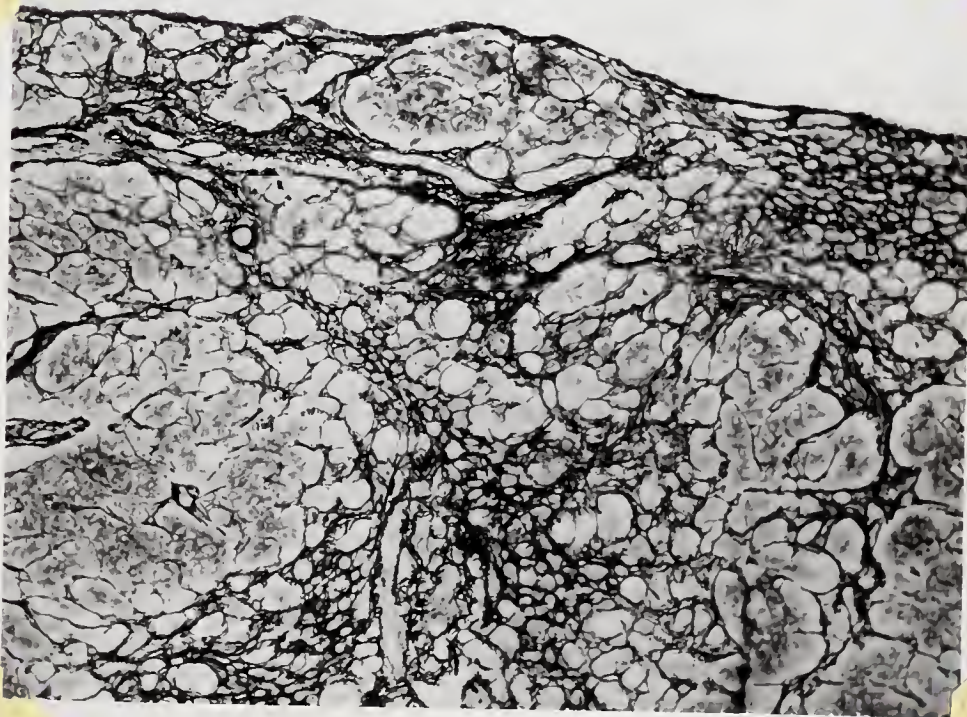


Fig. 146

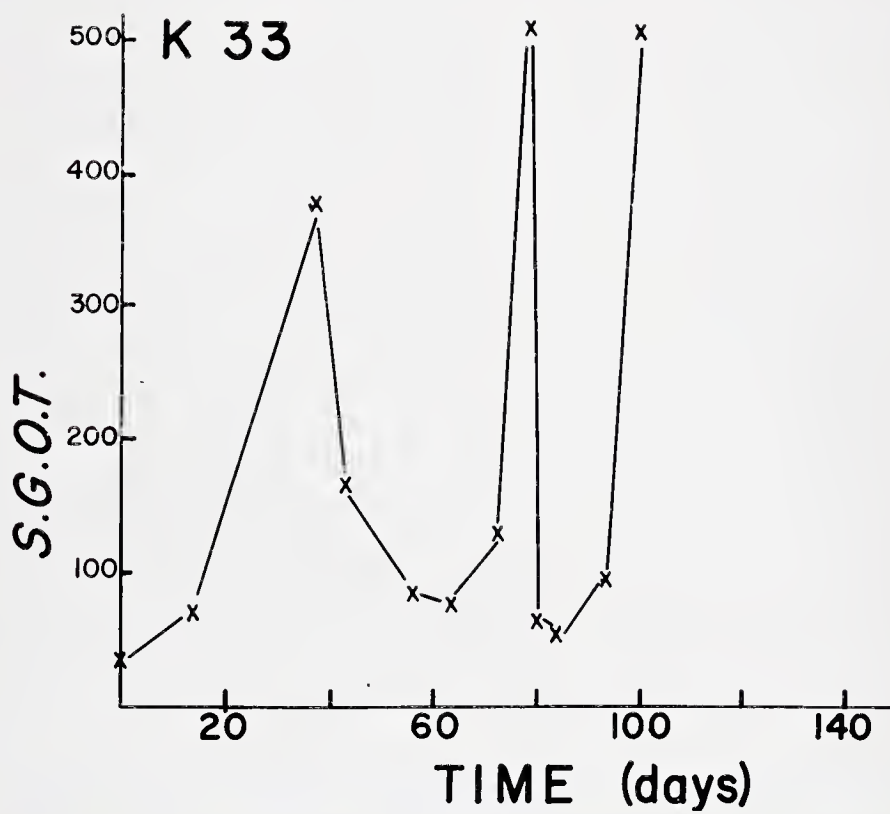


Fig. 147

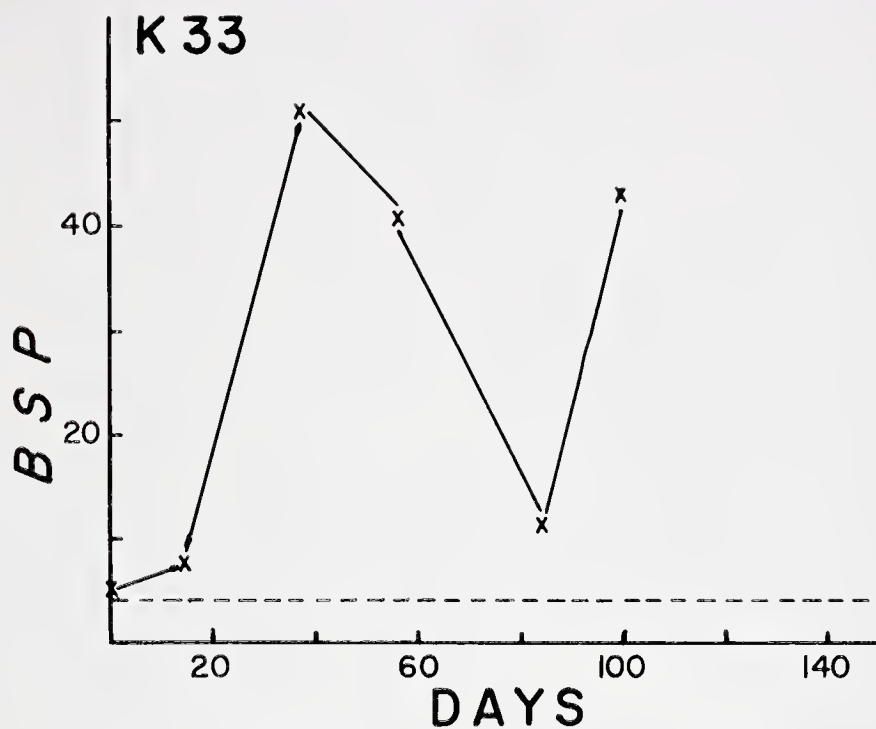


Fig. 148

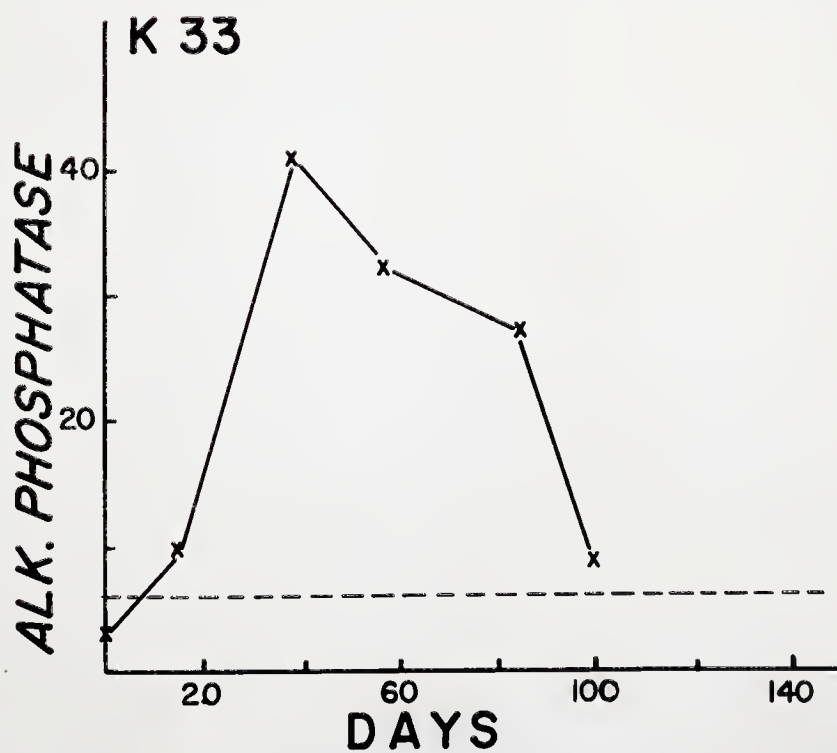


Fig. 149

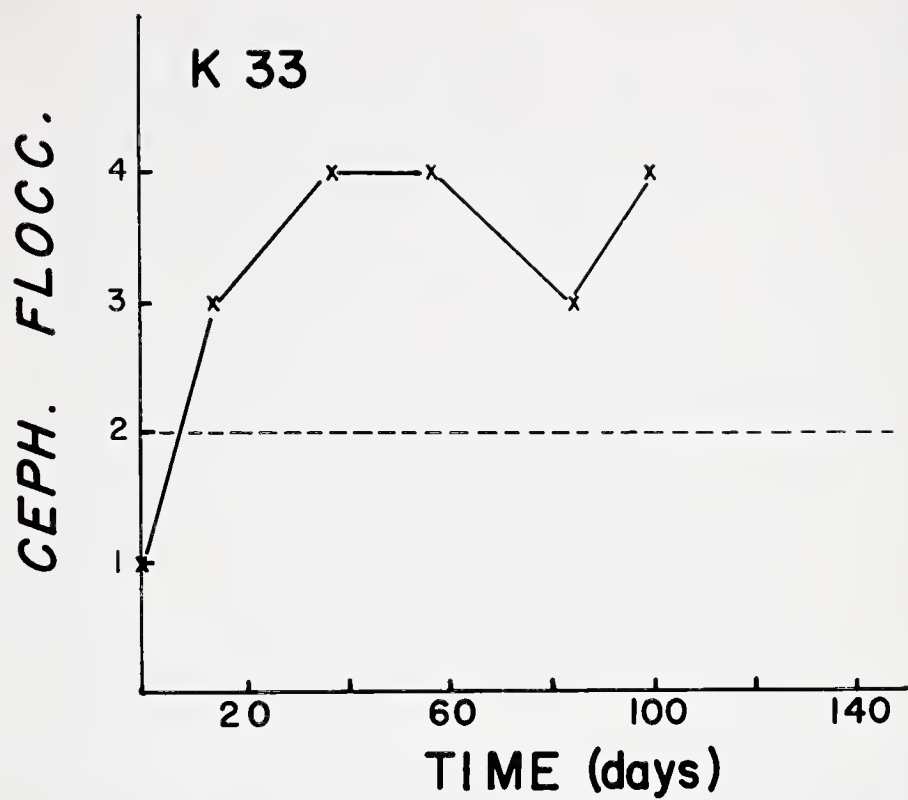


Fig. 150

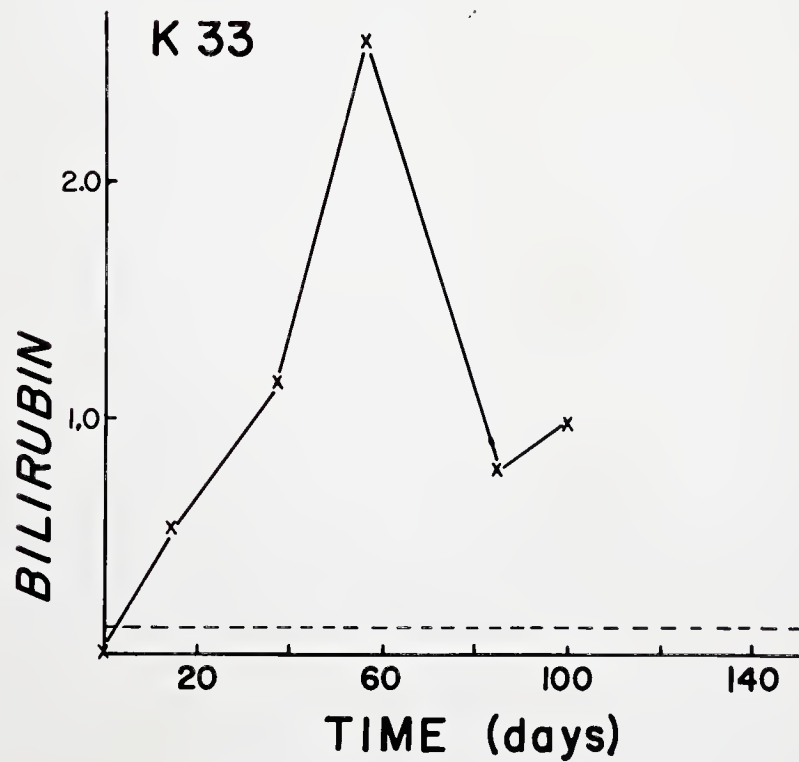


Fig. 151

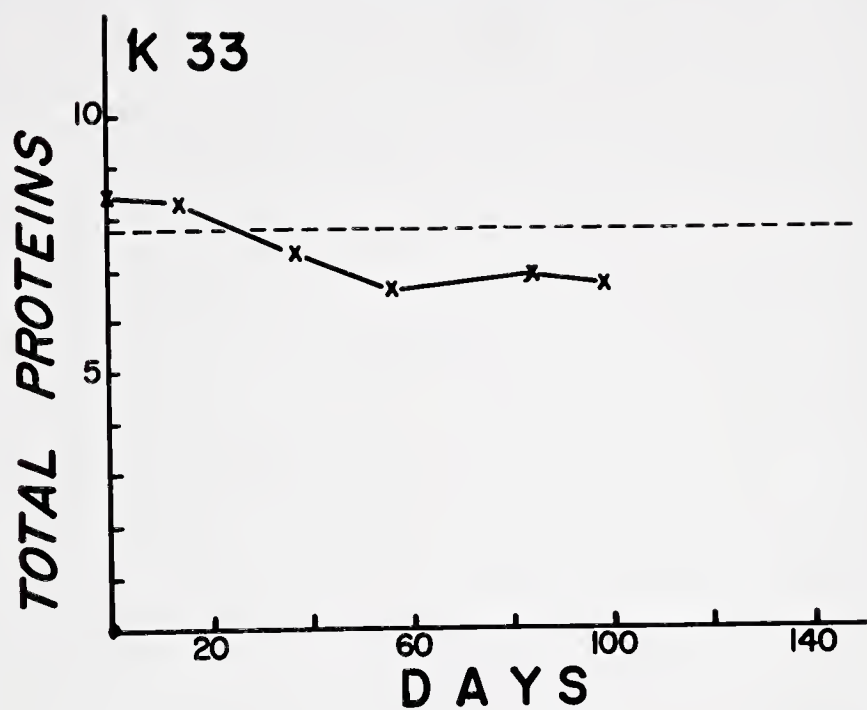


Fig. 152

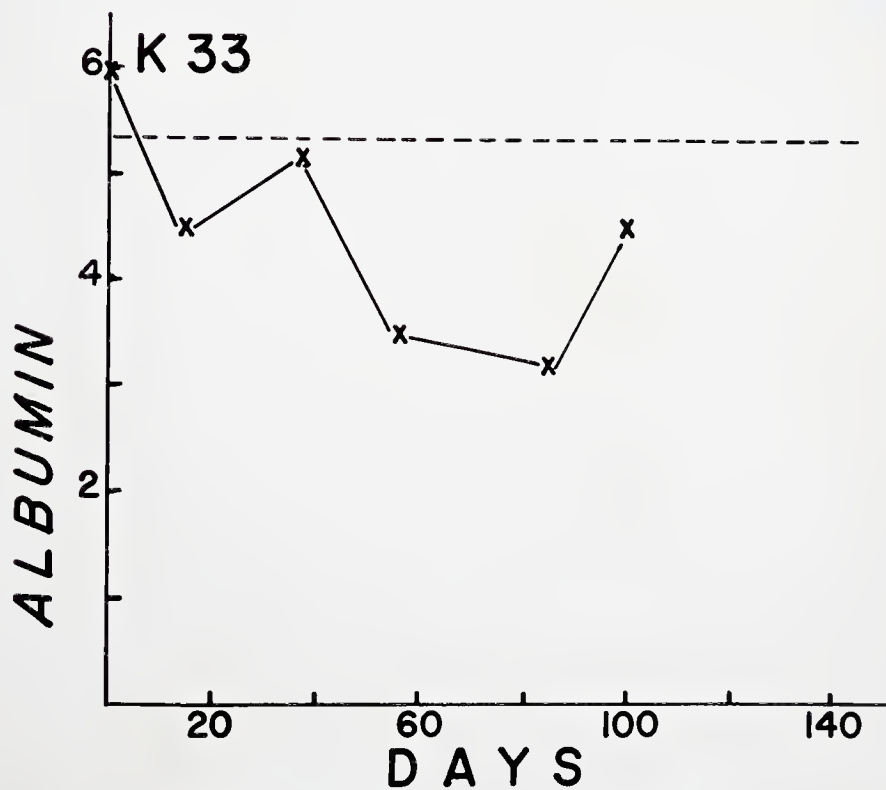


Fig. 153

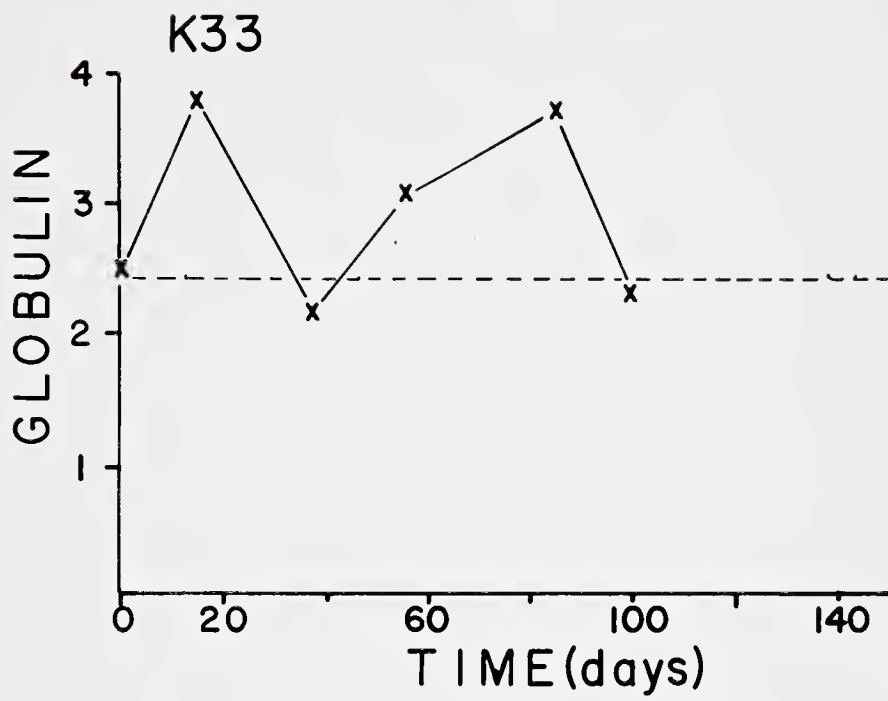


Fig. 154

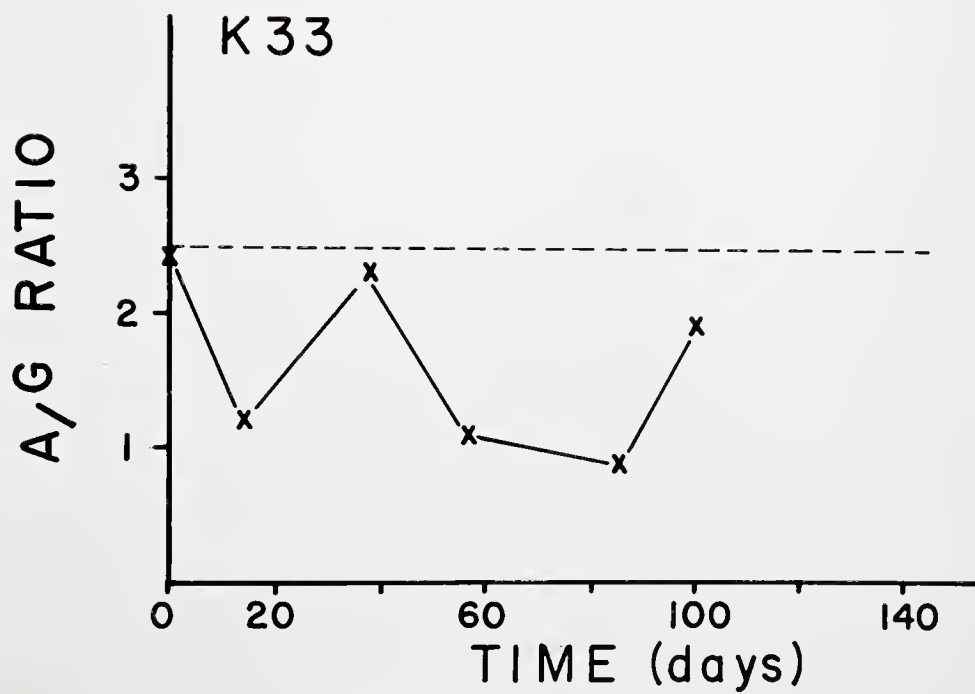


Fig. 155

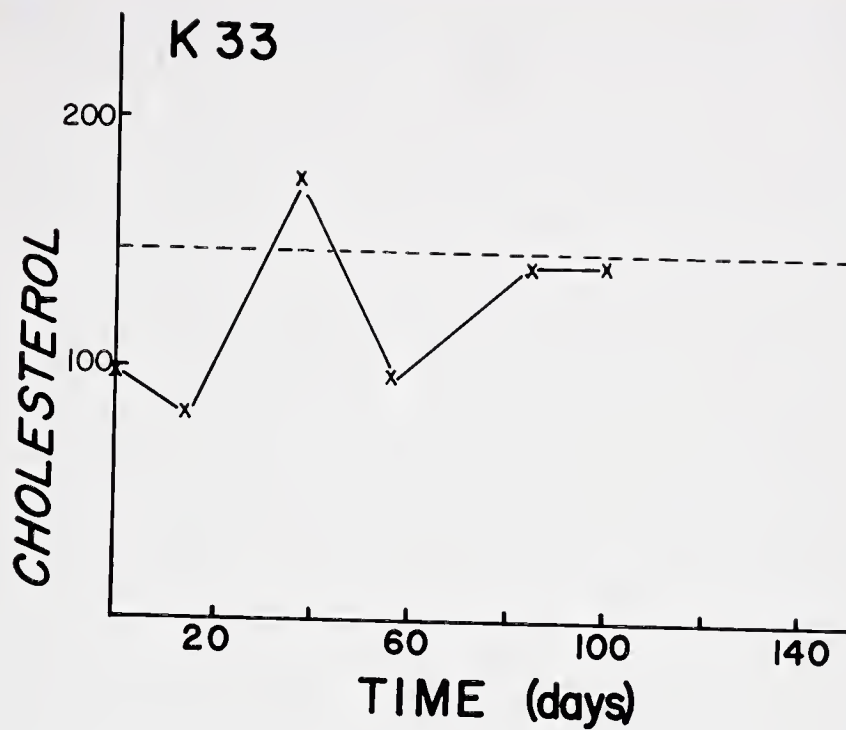


Fig. 156

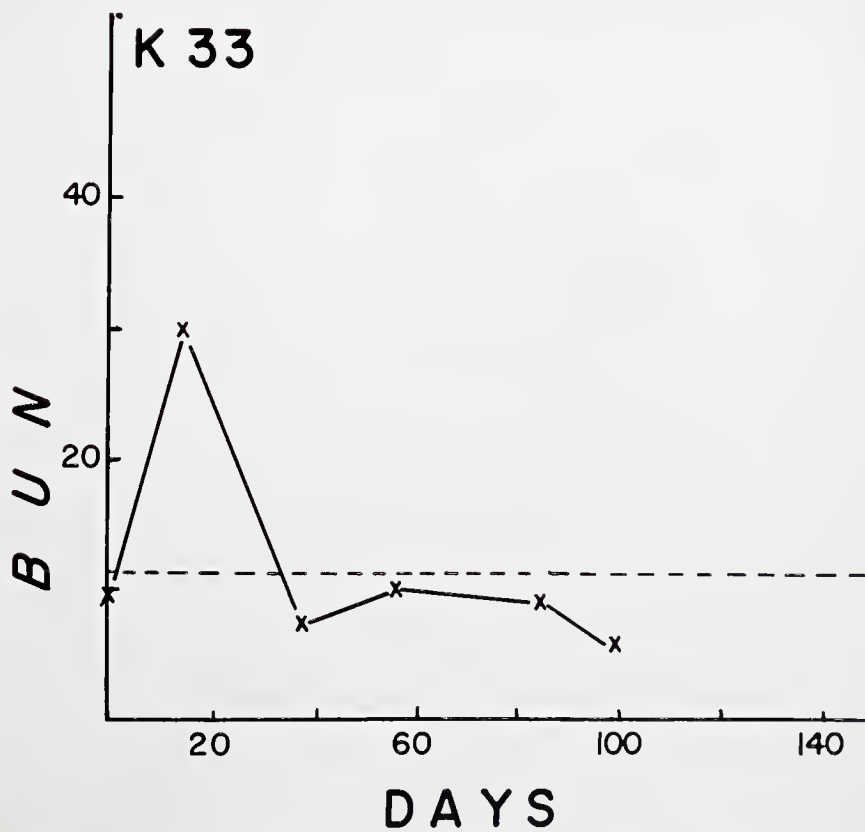


Fig. 157

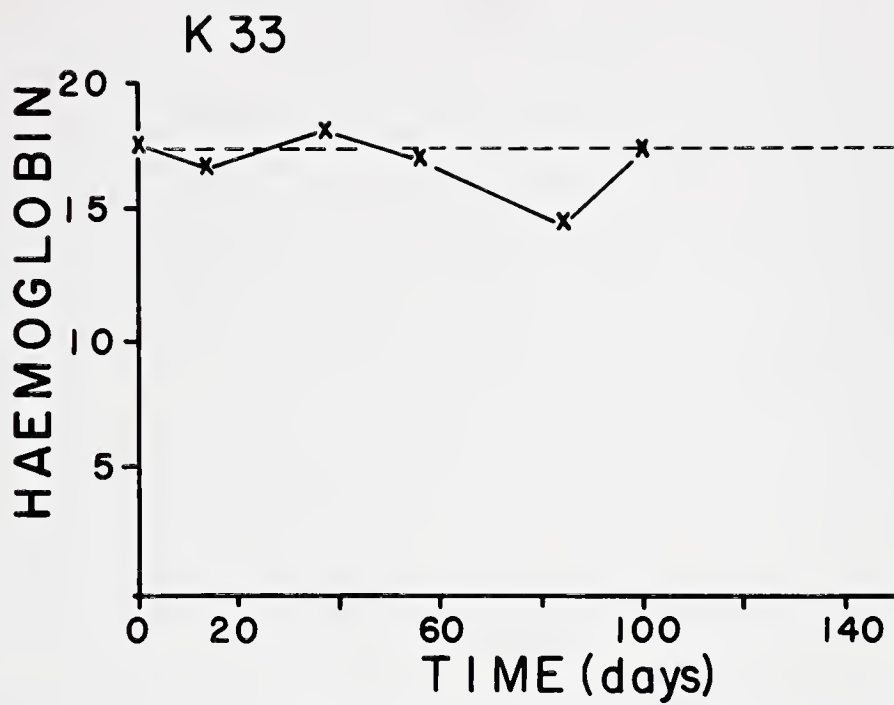


Fig. 158

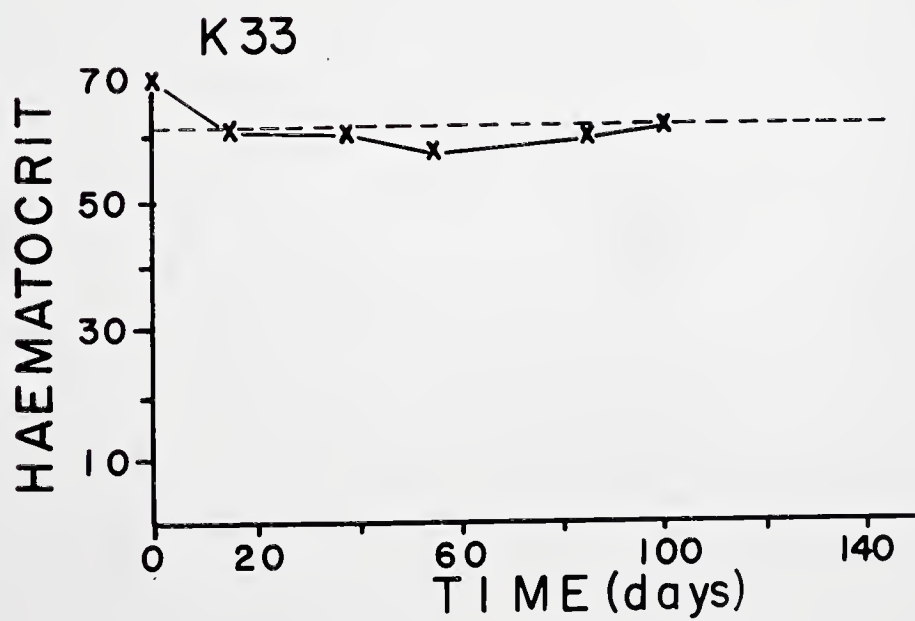


Fig. 159

DOG K-34

Hematological and biochemical investigation on the 4th of January, 1960, revealed no abnormality of liver function. Clinically the animal was alert, fit and healthy. Oral feedings with carbon tetrachloride in gelatin capsules commenced on the same date following the extraction of blood for testing.

Initially the animal was given 2.25 ml. carbon tetrachloride every second to third day, the dose being gradually increased until at the time of laparotomy on the 8th of April, 1960, 95 days after the start of the experiment, 10 ml. carbon tetrachloride were being administered on alternate days. An attempt was made to regulate the dose of carbon tetrachloride by intermittent SGOT estimations, but in this animal it proved exceedingly difficult, as reference to the graph on SGOT levels clearly demonstrates.

This was interpreted as with the preceding dog K-33, to indicate an unpredictable reaction to the toxin, for the dose was only gradually increased.

Except for one period, following the nasogastric intubation of 15 ml. carbon tetrachloride under Nembutal anaesthesia on the 15th of March, 1960, the dog

remained clinically healthy, although there was a loss of 4.5 kg. in weight during the experiment.

Following the nasogastric intubation of carbon tetrachloride the animal remained unconscious for 48 hours, after which it was drowsy for a further 48 hours. By the 27th of March, 1960, the dog appeared quite well again. This episode was interpreted as an acute instance of Barbiturate poisoning, due to the inability of a malfunctioning liver to detoxify the anaesthetic agent. During the period of unconsciousness the animal was treated as an acute Barbiturate poisoning in the following manner. Picrotoxin and Megimide were given intravenously intermittently in the prescribed dosage, which was modified for the animal's weight. An intravenous transfusion of 5% Dextrose in saline was given continuously during the period of unconsciousness.

During the 95 days of the experiment the dog received 218.75 ml. carbon tetrachloride via the oral route.

FINDINGS

The liver was normal in size, exhibited a nodular surface and was of a reddish brown color. The nodules were small, measuring 1 to 2 mm. in diameter. On palpation the consistency was rubbery, a character-

istic which was confirmed by incisional biopsy.

Ascites was present in a mild degree, the clear straw colored fluid filling only the paravertebral and iliac fossae.

The spleen appeared normal and there was no evidence of an increased portal systemic collateral circulation. No other abnormality was noted.

The portal pressure measured 210 mm. of saline.

HISTOPATHOLOGY

This specimen is particularly interesting in that it presents a composite picture representative of both diffuse septal and postnecrotic cirrheses.

The hepatic architecture is grossly obscured. In place of the usual lobular pattern is a disorganized picture of a diffuse increase in connective tissue associated with numerous and diffusely situated regenerative nodules.

Finger-like processes of connective tissue interdigitate themselves throughout the parenchyma. In some areas the fibroblasts appear healthy and active while in others, the appearance of broad sheets of fibrosis are suggestive of collapse and compression of necrotic cells. "Passive" septums of this nature are particularly obvious at the periphery of regenerative nodules and this is clearly seen in the reticulum stained sections.

A marked increase in the number of bile ducts is present, as well as a diffuse increase in inflammatory cells, mainly monocytes and histiocytes.

The vascular pattern is grossly irregular and it is with difficulty that patent central veins are recognizable.

In some areas there are large segments of collapsed cells separating irregularly sized regenerative nodules. In these areas there is not much evidence of an increase in "active" connective tissue, while the "passive" variety is abundant.

The presence of two of the major varieties of cirrhosis in one specimen is highly interesting and would tend to support the hypothesis that cirrhosis is a pathological state of the liver which varies with numerous factors of which only one is the main etiological cause. That is to say, a liver is capable of varying its response to a cirrhosis-provoking factor according to numerous other factors, each of which is capable of directing the pathological response to some degree.

It could be postulated further in this particular animal, that the mixed response was related to the

fact that it was exceedingly difficult to monitor the amount of necrosis produced by the SGOT estimations. This might indicate that the dog's liver was unduly sensitive to carbon tetrachloride and that this sensitivity varied, resulting in submassive necrosis on occasions, while on others, degeneration of cells only occurred. (See Figs. 159 - 164).

BIOCHEMISTRY

Again the SGOT level was most difficult to maintain around 100 units. On many occasions the level was over 200 units, approaching 500 units on two occasions. However, apart from the episode following Nembutal anaesthesia, the animal maintained a fairly good clinical health although there had been a gradual decrease in weight.

The BSP retention rose early in the experiment and maintained an abnormally raised level throughout.

Similar findings were present for the Serum Alkaline Phosphatase, Serum Bilirubin and the Cephalin Cholesterol flocculation.

In all the above mentioned parameters except the last, there was a moderate fall following the initial elevation of their levels. Again there was a tendency in these parameters to imitate the pattern exhibited by

the SGOT graph.

Both Serum Cholesterol and BUN estimations demonstrated a fluctuation within the limits of normality.

The results of the Serum Protein estimations were similar to those described in the majority of the dogs in this experiment. There was a gradual decrease in the Total Serum Proteins with a concomitant rise in Serum Globulin and fall in Serum Albumin. The Albumin-Globulin ratio diminished gradually until it was reversed. (See Figs. 165-175).

HEMATOLOGY

Both the Hemoglobin and Hematocrit estimations demonstrated a gradual fall but remained nevertheless within the limits of normality. (See Figs. 176, 177).

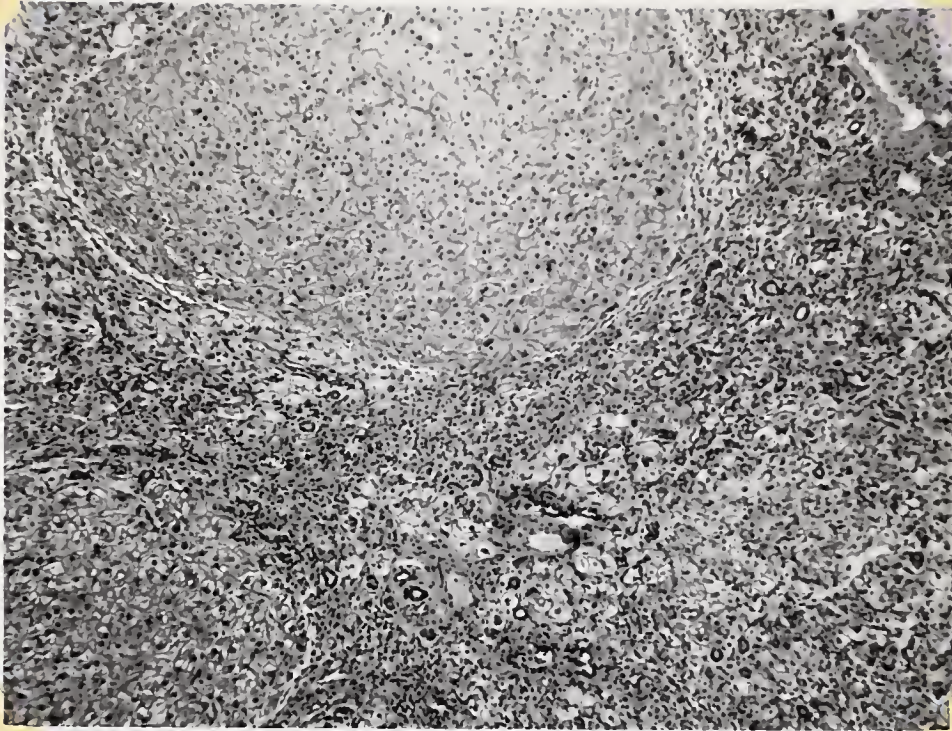


Fig. 160

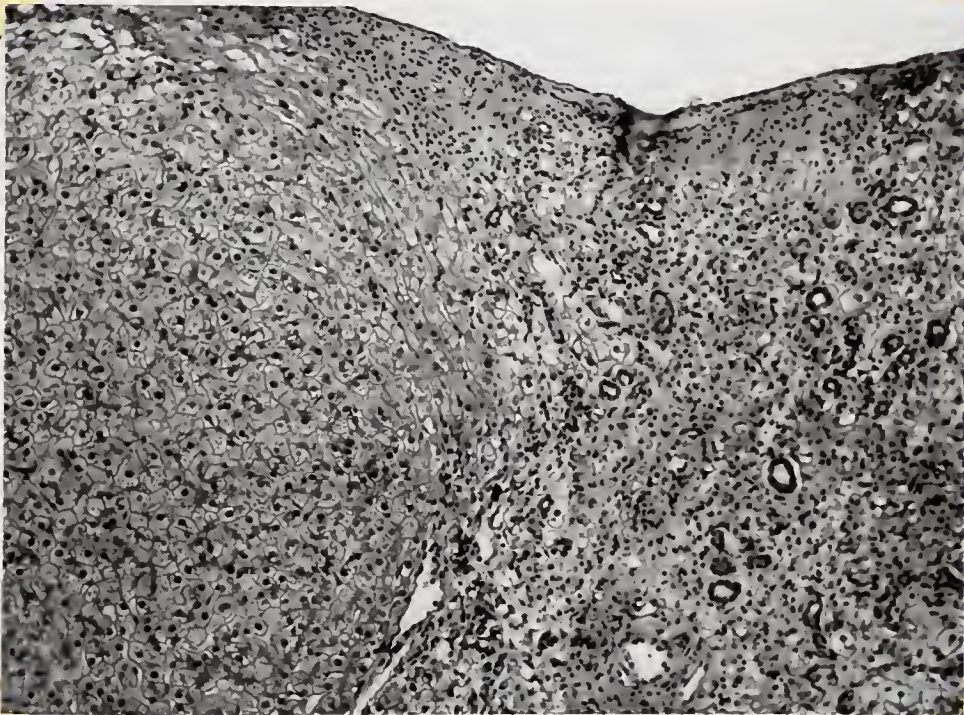


Fig. 161

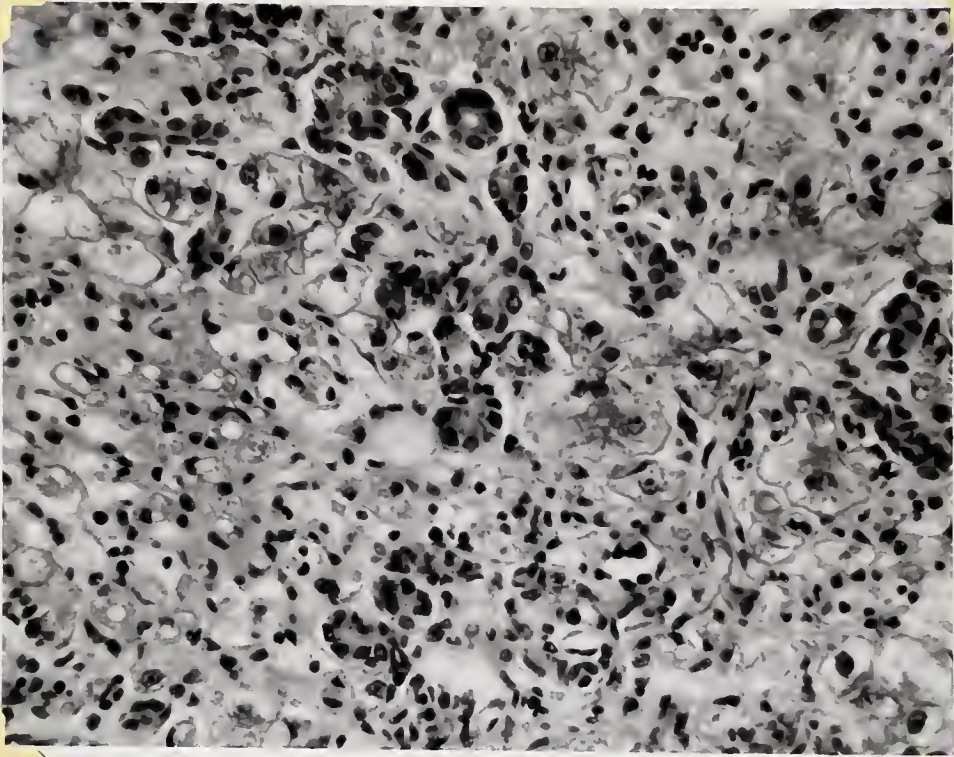


Fig. 162

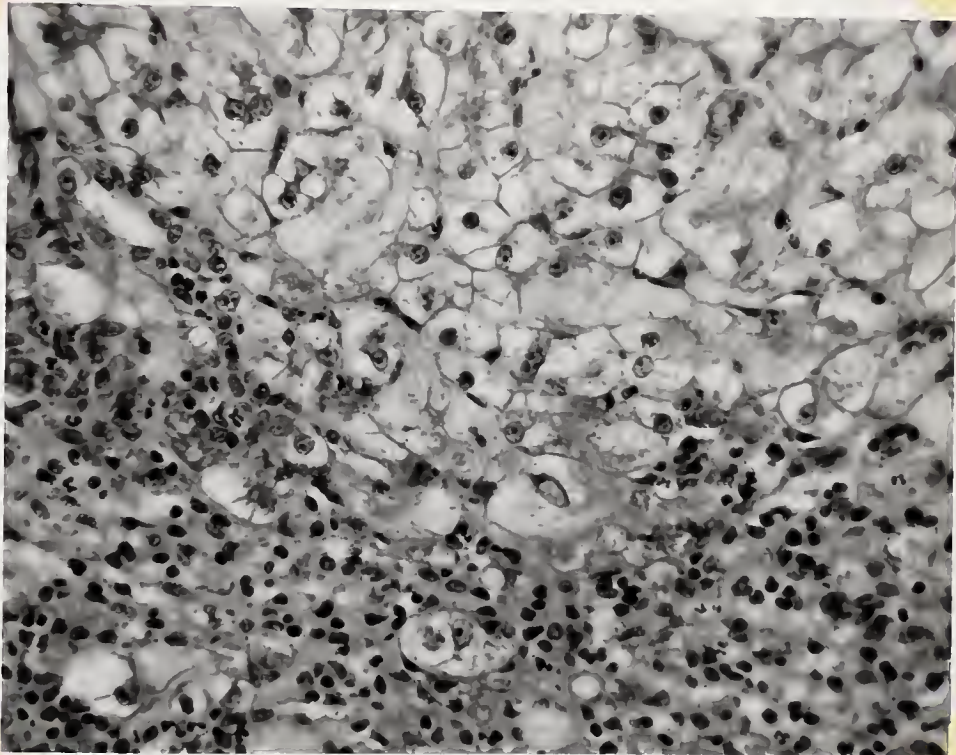


Fig. 163

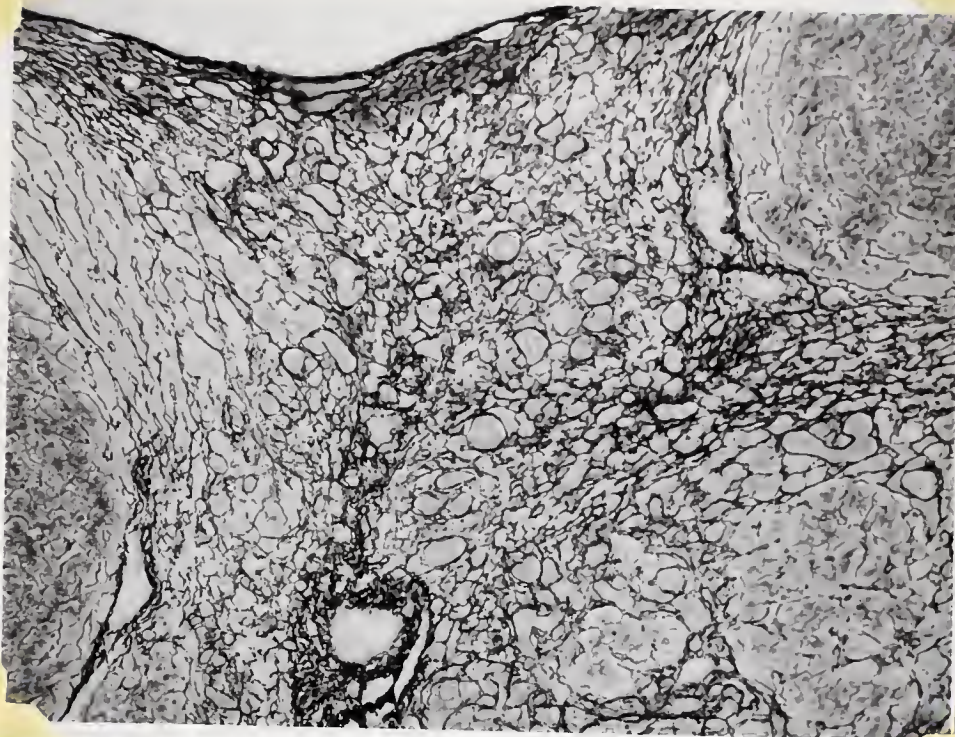


Fig. 164

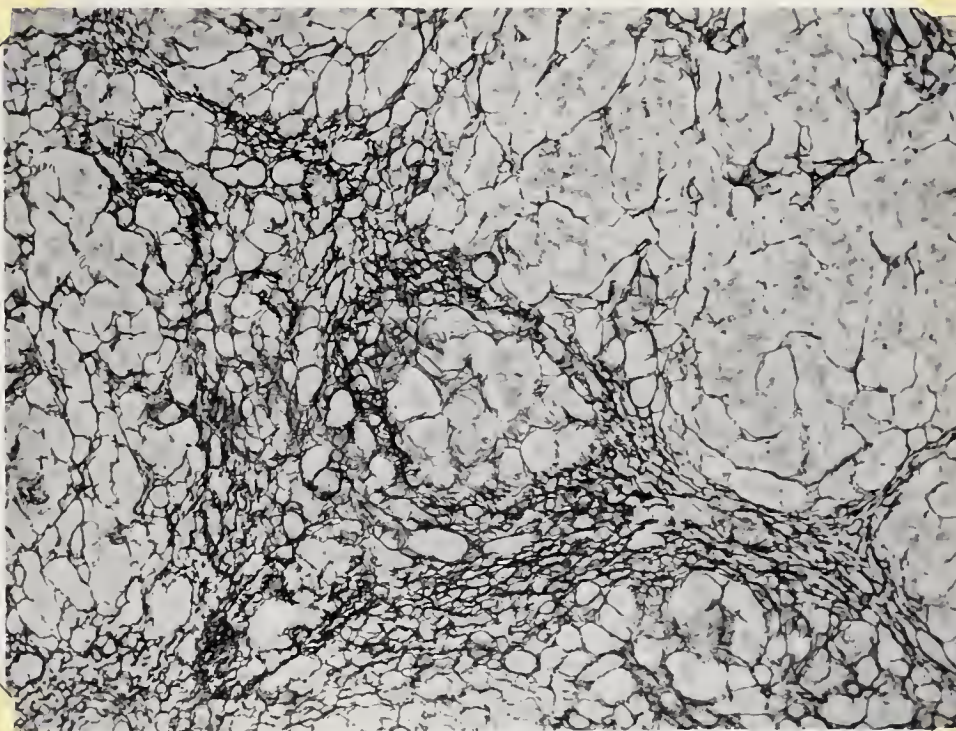


Fig. 165

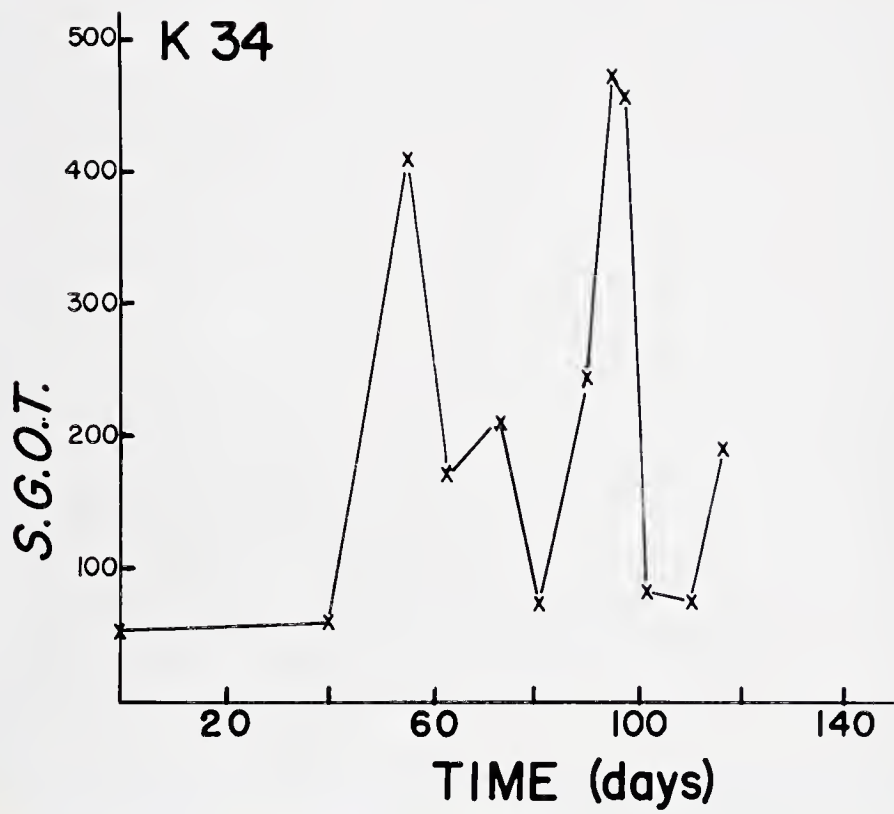


Fig. 166

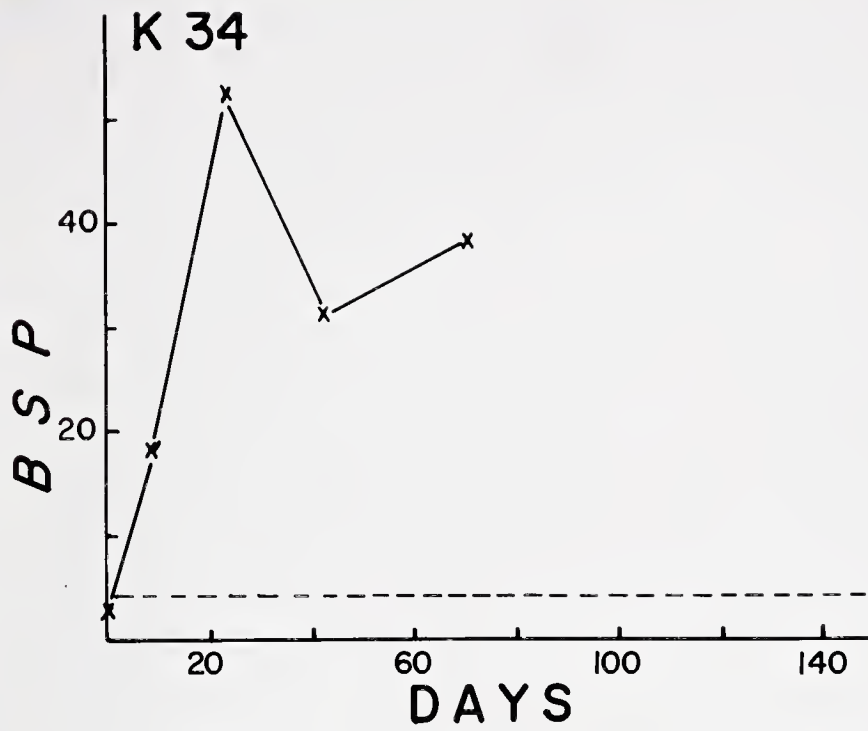


Fig. 167

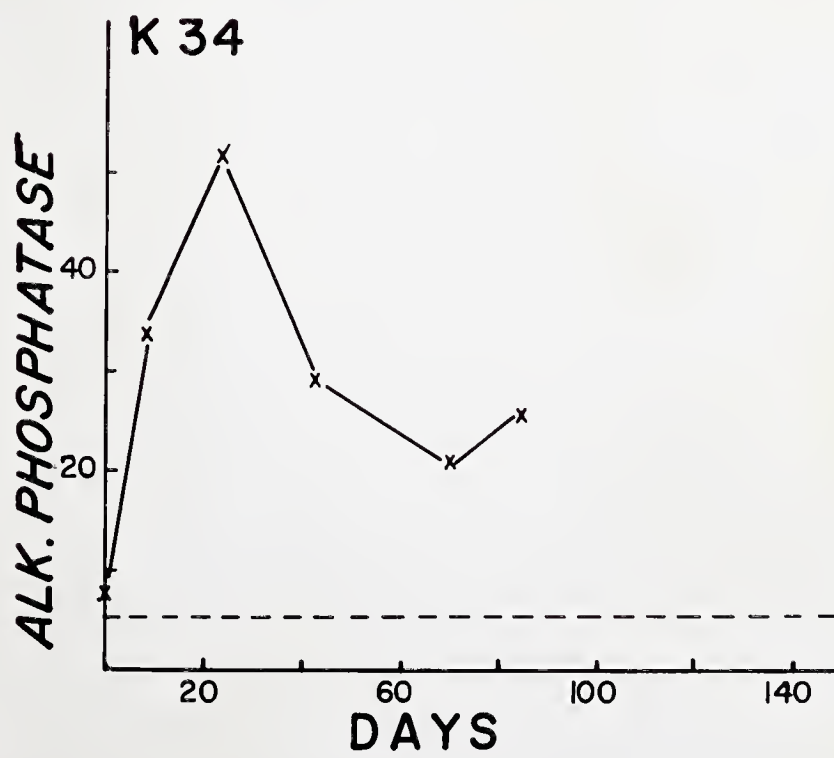


Fig. 168

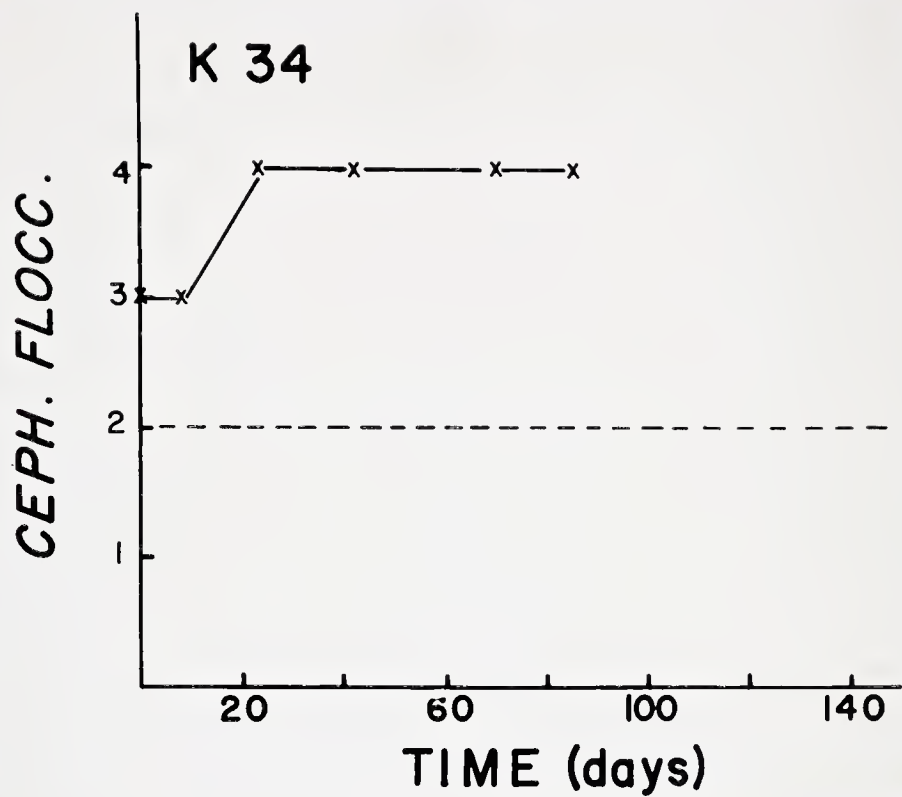


Fig. 169

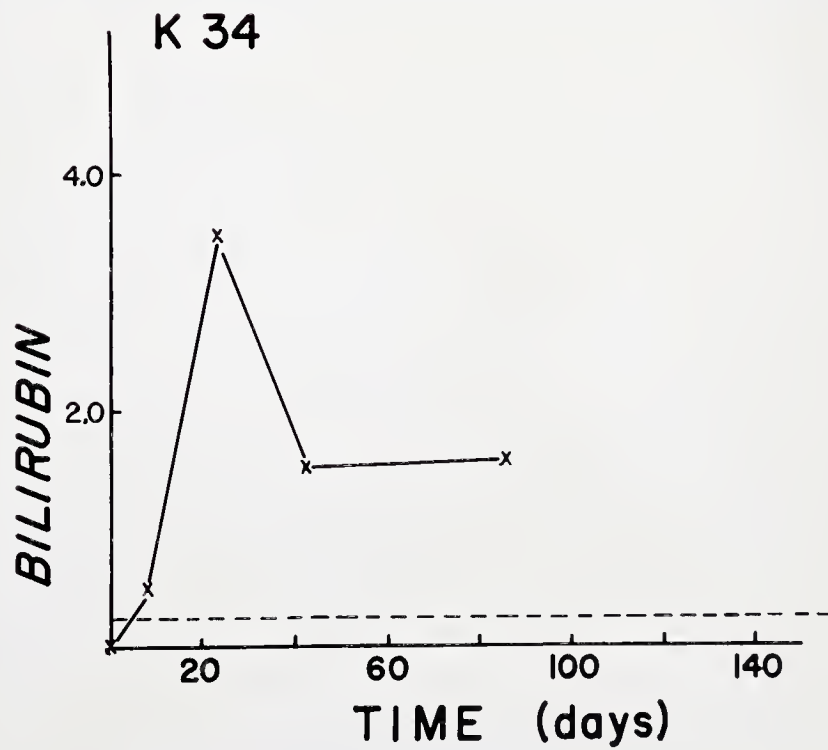


Fig. 170

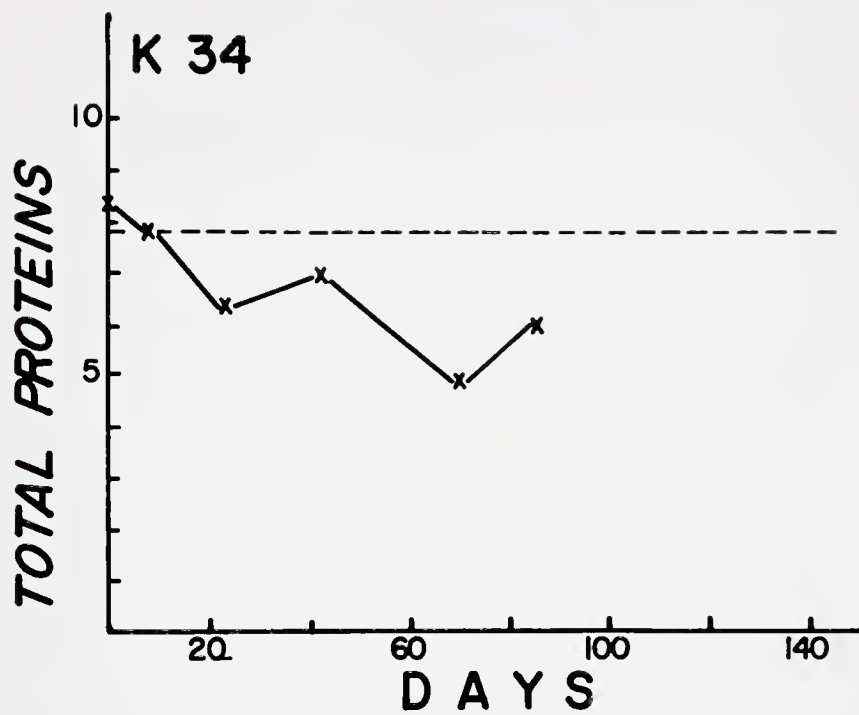


Fig. 171

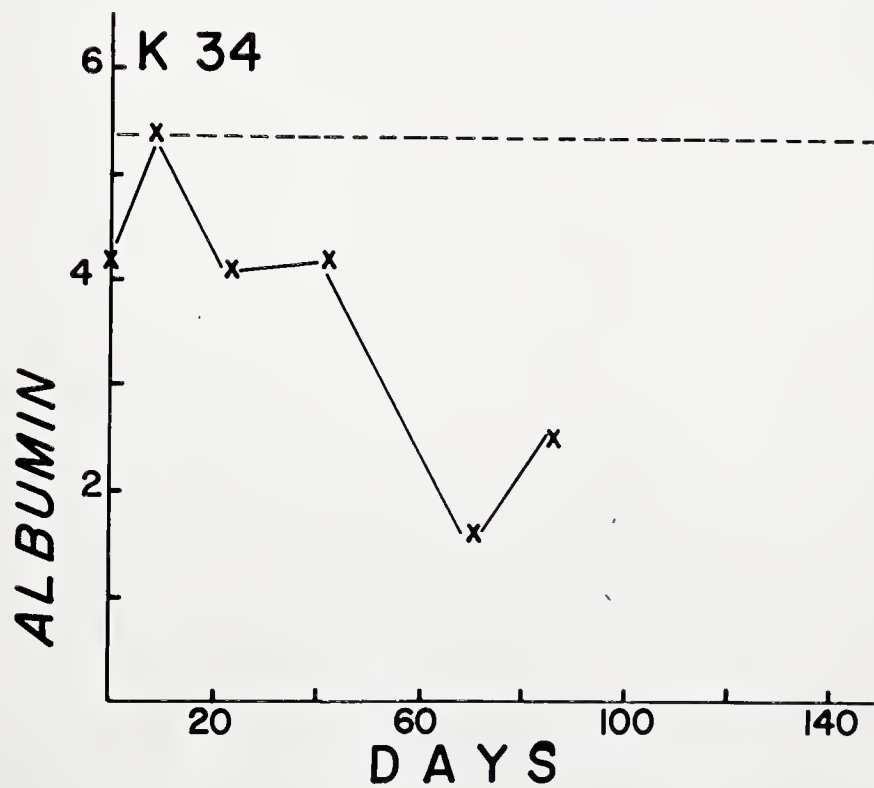


Fig. 172

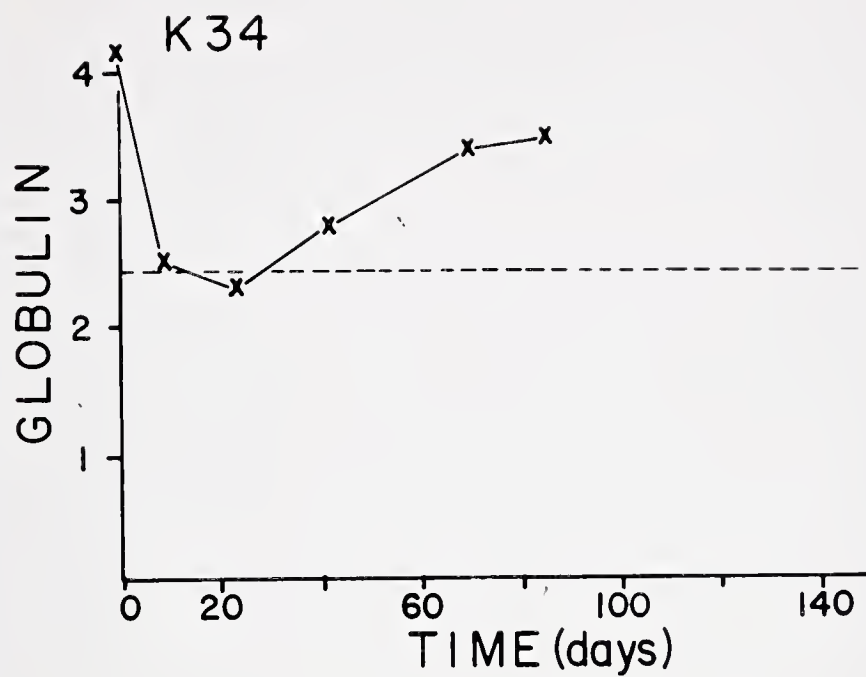


Fig. 173

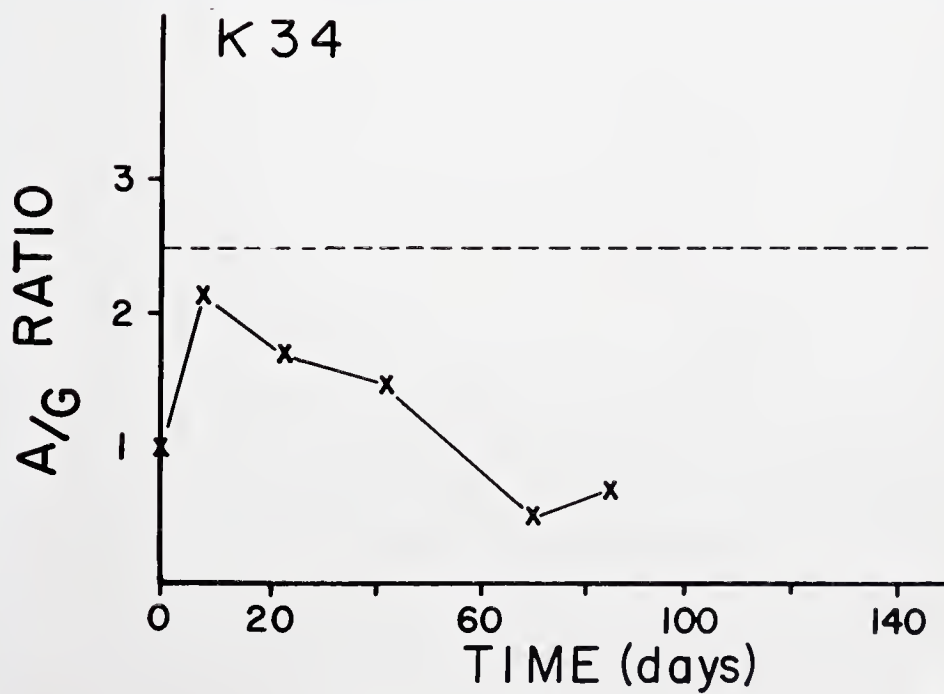


Fig. 174

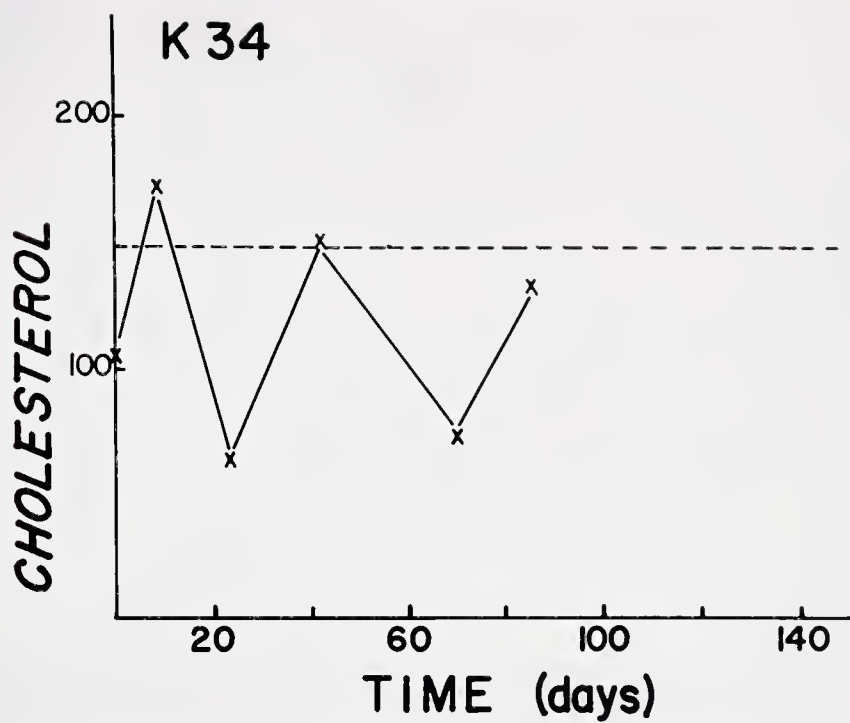


Fig. 175

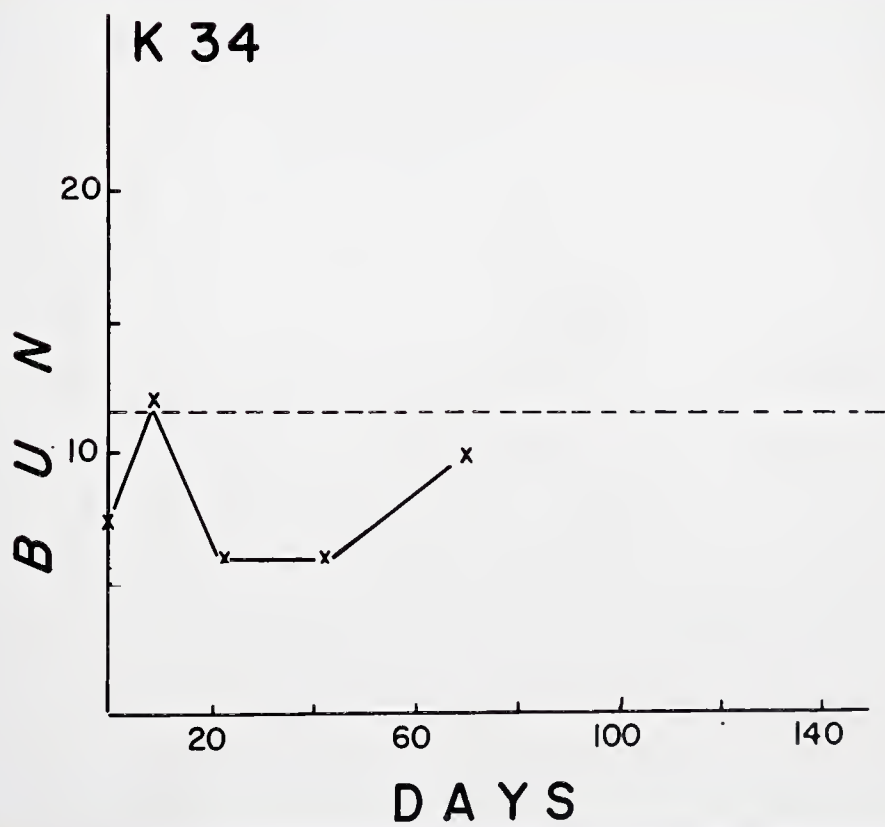


Fig. 176

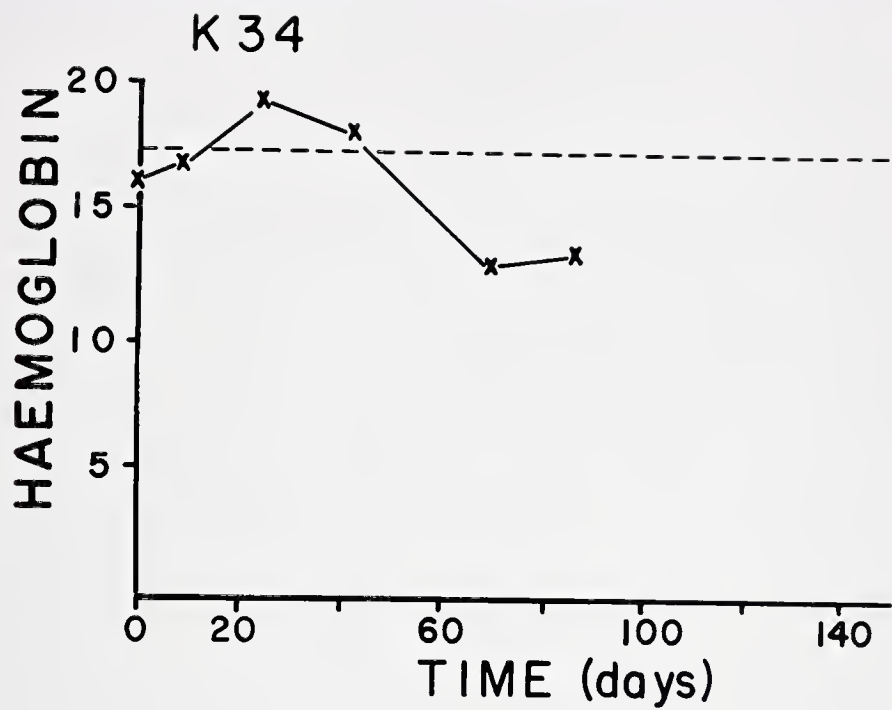


Fig. 177

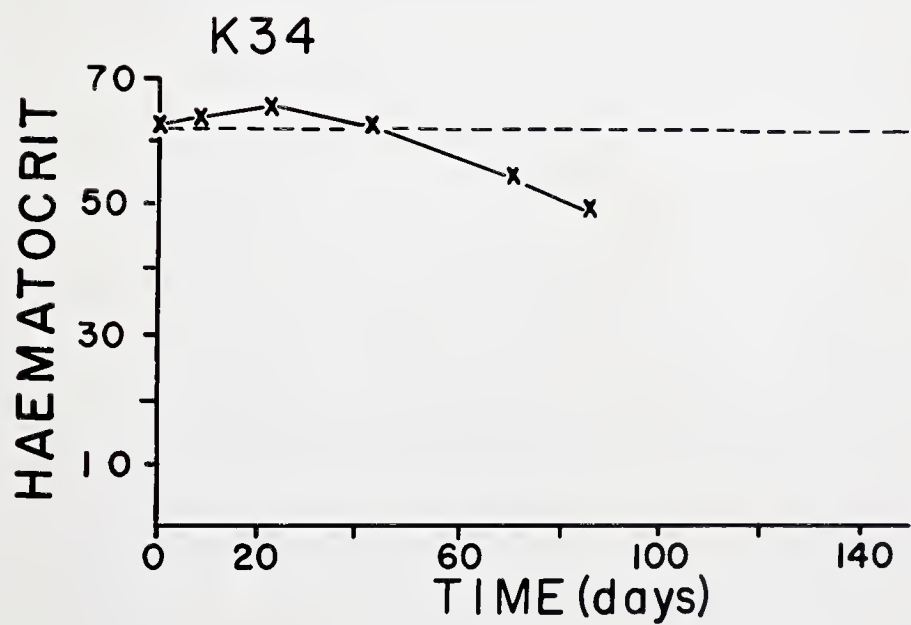


Fig. 178

CHAPTER III

DISCUSSION OF RESULTS

Ten dogs were fed carbon tetrachloride intermittently in gelatin capsules over a period ranging from 87 to 139 days. Five of the animals developed a diffuse septal or portal cirrhosis, and three demonstrated a mixed form of the disease in which both portal and postnecrotic forms were present. The remaining two dogs in the series exhibited a diffuse degeneration of the hepatic parenchyma associated with cellular collapse, some disfiguration of the liver architecture and occasional increase in connective tissue. A diagnosis of cirrhosis was not justified in these two animals, but it seemed that the pattern of the pathological changes suggested the description of a pre-cirrhotic stage of the disease.

The coexistence of portal and postnecrotic forms of cirrhosis in the same specimen is extremely interesting and would tend to support the hypothesis that different forms of cirrhosis may be produced by the same etiological agent. It may be argued further that cirrhosis is simply the image of a pathological process at a certain point in its genesis, which is constantly in a state of flux dependent on various other factors including (1) strength or virulence of the

etiological factor, including its mechanism of action, (2) the manner in which it enters the organism, (3) the rate at which it enters or affects the organism, (4) the period and periodicity of its entering or affecting the organism, (5) the diet and (6) the state of the liver at the time of its exhibition to the etiological factor.

Three of the dogs developed ascites and in the eight dogs in which laparotomy was performed, the portal pressure was significantly elevated.

As a result of the biochemical investigations significant changes were found to occur in the SGOT, Serum Alkaline Phosphatase, Serum Bilirubin, BSP retention, Serum Proteins and Cephalin Cholesterol flocculation. Changes in the Serum Cholesterol were not significant, while findings with the BUN bordered on significant.

Changes in the Hemoglobin level were not significant while those of the Hematocrit were just significant.

The condensed results of all the biochemical investigations over the entire period of the experiment are presented as histograms in Figs. 180-192. The results of the Mesenteric Pressures are shown in Fig. 181. The total dosage of Carbon Tetrachloride administered to each dog over the entire experimental period is also included in each histogram.

The results are presented in detail on Table #2 in which they are compared with the normal average results found in 26 healthy dogs.

It would appear therefore from the results obtained in this experiment that certain parameters of liver function alter significantly in a certain direction during the pathogenesis of carbon tetrachloride induced portal cirrhosis, and that in some instances the changes relate directly to the degree of hepatocellular necrosis as indicated by the level of SGOT.⁽⁵⁷⁾

This alteration is interpreted as a change in liver function, a degree of hepatic dysfunction. A function test is useful in both indicating and establishing the existence of an abnormality as well as measuring the extent of the abnormality. Zieve⁽⁷⁶⁾ in his paper on "The Apparent Meaning of Liver Function Tests", discusses four kinds of information concerning hepatic tests that one must have in mind for the proper interpretation of recorded values.

1) Explicit knowledge of the laboratory procedure, since a given test is influenced by minor variations in laboratory procedure. As an example the BSP retention test is cited, which is influenced by the use of protein precipitants or by corrections for hemolysis.

2) Awareness of extraneous factors which may influence the values obtained with the test. For example the BSP retention test is influenced by body weight and the degree of jaundice. The percentage of cholesterol substance in the blood serum is likewise influenced by the degree of jaundice.^(7 7) The reduction in percentage of cholesterol substance in the presence of liver disease is dependent to a large extent upon the degree of jaundice present, and to a lesser extent upon the degree of hepatocellular dysfunction. The changes in Serum Cholesterol in this experiment were not significant but when this is correlated with the fact that the degree of jaundice produced in the dogs was not marked, this is in agreement with Zieve's findings.

3) Recognition that the normal limits of the tests are often appreciably higher than most of the normal values one generally sees because of the skewness of the distributions.

4) Valid limits of normal values for the tests. To satisfy this criterion one would have to distinguish a zone of doubtful or questionable abnormality and a zone of definite abnormality.

It is agreed that the above four kinds of information must be kept in mind when interpreting the data obtained in this type of project, and for this

reason all tests were carried out by the same technician using the standardized techniques described earlier in this presentation.

Liver function tests are used to demonstrate an abnormality of a particular function of the liver. These include biochemical hepatic abnormalities referable to -

- 1) Bile pigment metabolism.
- 2) Excretory function.
- 3) Detoxification and conjugation.
- 4) Metabolism of protein, carbohydrate and fat.
- 5) Specific enzymes.
- 6) Storage of minerals and vitamins.
- 7) Regulation of hormones.
- 8) Water and electrolyte balance.

Broadly speaking the liver function tests are an indication of synthesis or excretion or the combination of both as, say, in the level of Serum Bilirubin, which indicates the rate of excretion of Bilirubin as well as the rate of synthesis of Bilirubin into glucuronide prior to excretion, which must affect the serum level.

It must be emphasized, however, that no single hepatic function test is indicative of the capacity of the liver. Current hepatic function tests are

too nonspecific and not sufficiently quantitative to act as an accurate standard index. Some investigators feel that liver function tests reflect hepatocellular changes fairly well,^(11,78) while others emphasize the lack of correlation.

The liver probably has several hundred specific physiological activities and only a rough correlation exists between morphological and biochemical alterations in diseases of the liver. The fact that regeneration continues simultaneously with the morbid forces of inflammation, necrosis and fibrosis, adds further to the complexity of assessing the degree of liver damage present. The untoward effects of the disease on liver function may be more than offset by the remarkable regenerative powers of this organ.

The various hepatic function tests used in this experiment will now be discussed separately in an attempt to correlate them with increasing liver pathology as evidenced finally by laparotomy and liver biopsy.

1. Serum Bilirubin

Impaired bile pigment metabolism which occurs in cirrhosis results in an increase in the Total Serum Bilirubin. This may reflect either hepatocellular damage, obstruction of either the intrahepatic or

extrahepatic bile ducts or in the case of secondary hypersplenism, increased hemolysis or erythrocytes. One is impressed with the lack of correlation between histological evidence of hepatic damage and the amount of fractionated Serum Bilirubin. (73,79)

This parameter of liver function, as mentioned above, indicates both rate of excretion and synthesis of bilirubin.

In all ten dogs there was a gradual and early increase in Serum Bilirubin. Only in K-31 was the increase slight and not maintained. There appeared to be a proportional relationship between SGOT and Bilirubin levels, reflecting in the first incidence hepatocellular damage and secondly obstruction due to the edema following the hepatocellular necrosis. There did not however appear to be any correlation between the level of Serum Bilirubin and the total quantity of carbon tetrachloride administered, or the length of the period during which the toxin was administered. In this experiment the Serum Bilirubin has proved to be a moderately accurate indication of hepatic dysfunction.

2. B.S.P. Excretion Test

The excretory function of the liver is determined by its ability to excrete foreign dyes, of which Sulfobromophthalein is one of the most sensitive.

Unfortunately exogenous material is required and the exact nature of the excretory process is not well defined. Seligson⁽⁸⁰⁾ believes that some energy requiring process is involved in dissociating BSP away from Serum Albumin; getting it into the liver parenchyma and out through the biliary tree. In some regards, this test also reflects synthesis as well as excretion. There was no correlation between the total amount of carbon tetrachloride administered, the period of administration and the level of BSP retention. However, in all the dogs, this parameter became elevated early in the experiment and remained significantly raised throughout the period of administration of the toxin. There was a definitely proportional relationship between the SGOT level and the BSP retention during the period of the experiment. This would indicate a close correlation between BSP retention and hepatocellular damage. It is also a sensitive test of biliary stasis and circulatory impairment in the liver, but there is no way of accounting for the degree to which each of these pathological conditions contribute to the elevation of this parameter of liver function. Retention of BSP is found regularly in patients with cirrhosis, but there is only fair correlation with morphological damage of the liver.^(71,72) This was found to be true in this experiment.

3. Serum Proteins

The synthesis of proteins is one of the most important functions of the liver as evidenced by the changes in the various serum proteins; for example, Prothrombin, Fibrinogen, Albumin, Globulin and certain proteinases during hepatic disease. Cirrhosis may be reflected by abnormalities of the various protein fractions in the serum but electrophoretic patterns do not usually distinguish the type of cirrhosis. Elevation of the Serum Globulins in cirrhosis is thought to reflect stimulation of the reticuloendothelial system.

During the course of this experiment there was a tendency for the Total Serum Protein level to decrease. Statistically this was found to be significant for the group. There was also a significant decrease in Serum Albumin and the Albumin-Globulin ratio. The Serum Globulin demonstrated a gradual increase but this was not found to be statistically significant. What was interesting was the fact that the three animals which developed ascites demonstrated the greatest fall in the Total Serum Proteins, Serum Albumin and the Albumin-Globulin ratio, while the Serum Globulin was raised to a greater level than in the other dogs. These tests appeared to demonstrate a fairly accurate and significant indication of liver function.

4. Cephalin Cholesterol Flocculation Test

This is another test of abnormal protein metabolism, though not specific for hepatic disease. The cephalin Cholesterol Flocculation test determines qualitative amounts of flocculation of serum upon the addition of a cephalin cholesterol commercial emulsion. It reflects an increase in the plasma content of albumin and alpha-globulin and is a fairly sensitive test of hepatocellular damage. The cephalin cholesterol flocculation test increased in all ten dogs. The average increase in these treated dogs was statistically significant.

5. Serum Cholesterol

The liver plays an important role in the metabolism of cholesterol. The level of Serum Cholesterol is usually raised in obstructive conditions of the intrahepatic or extrahepatic biliary tract, while hepatocellular damage is associated with a fall in the level of this substance. This may be useful in distinguishing a hepatocellular from an obstructive jaundice. Cholesterol is absorbed from exogenous sources and is also synthesized in the body, principally in the form esters which are degraded and excreted into the bile duct by the liver. Bile salts are synthesized in the liver from cholesterol.

Only two of the dogs, K-26 and K-27, both of which developed ascites demonstrated a gradual decrease in the level of serum cholesterol. The rest showed either an increase or no change. It would seem that concomitant hepatocellular damage and obstruction to the outflow of bile, which occurred, results in findings difficult to interpret. The average results in the treated dogs were not significant.

6. Serum Alkaline Phosphatase

The various biochemical activities of the liver including protein, fat and carbohydrate metabolism, are regulated by a great number of enzymes and co-enzymes about which very little is known. Alkaline phosphatase is one of the more well known of the hepatic enzymes, abnormalities of which may reflect hepatic insufficiency. The liver is one of the sources which both synthesize and excrete alkaline phosphatase into the biliary system and abnormalities of this enzymic level in the blood stream may reflect increased production within the liver, impaired excretion or a combination of these two abnormalities.

Clinically it is known to be markedly elevated in patients with intrahepatic or extrahepatic biliary obstruction and moderately increased with parenchymal hepatic damage.

In this experiment the dogs exhibited fairly early elevation of the Serum Alkaline Phosphatase which appeared to be related to the SGOT levels. The level remained elevated in all animals except K-31, K-32 and K-33 where it reverted to normal readings at the conclusion of the experiment. Except for K-31 this was not accompanied by a fall in Serum Bilirubin, suggesting a release of biliary obstruction, so it is difficult to explain. However, the average value for the ten dogs was markedly raised and statistically highly significant.

7. S.G.OT.

Among the newer tests which also reflect the integrity of the hepatic cell, is the SGOT. Much work has been done with this enzyme in relation to hepatic pathology.^(55,56,59,60) Transaminase is a specific enzyme concerned with the transfer of alpha-amino nitrogen of aspartic acid to alpha-ketoglutaric, resulting in the synthesis of a new amino acid, glutamic acid, and a new alpha-keto acid, oxalacetic acid.^(54,81) Its importance therefore in the Citric Acid Cycle of Krebs is obvious. Marked elevations of SGOT are found in conditions where hepatocellular necrosis has occurred. The SGOT level may be interpreted freely as an index of hepatocellular necrosis,

since this enzyme is released directly into the blood stream during conditions producing cellular necrosis. It must be remembered, however, that although the SGOT level depicts fairly accurately the state of hepatocellular necrosis, it may also be affected by inflammatory and traumatic conditions involving other portions of the anatomy particularly the muscular system. Due to its usefulness as an index of hepatocellular necrosis it was used in this experiment both to monitor the amount of carbon tetrachloride administered to each dog, and to maintain a knowledge of the extent of hepatocellular damage occurring so that the process would be gradual and the animals could maintain a moderate state of health. Human cirrhosis, as far as one knows, is not a disease of sudden occurrence so an attempt was made in this experiment to imitate its chronicity.

It was not possible to maintain a regular level of SGOT. The level fluctuated constantly, each necrotic assault on the liver being followed by a return to a normal level. An attempt was made to keep the SGOT level below 100 units, but this proved difficult. Frequently the level would change in a manner not commensurate with the quantity of the drug used.

The dogs all showed the ability to recover rapidly from the effects of an episode of hepato-

cellular necrosis if the carbon tetrachloride was either reduced or stopped temporarily. It was interesting to note that several of the parameters of hepatic function used, bore a proportionate relationship to the level of SGOT. These included BSP retention, Serum Alkaline Phosphatase and Serum Bilirubin.

The average value of SGOT for the 10 dogs during the period of the experiment was significantly raised, and in none was it below the average normal value.

8. Blood Urea Nitrogen

This is not strictly a test of liver function. It was used for two reasons. In the first place it is known that carbon tetrachloride may affect the kidneys, so this parameter was used to give some indication of renal function. A rise would have been interpreted as a measure of decreased renal function. On the other hand the BUN is intimately related to protein metabolism, an important property of hepatic function, so that this test could be used as an indirect indication of the state of protein metabolism in the liver as well.

The BUN results in this experiment suggest that kidney function is little, if at all, interfered with by oral carbon tetrachloride in the dosage used.

The results also suggest that they are a fairly accurate indication of protein metabolism in the liver, for the gradual decrease in Blood Urea Nitrogen correlated closely with the decline in the Total Serum Proteins. One point of interest is that initially in every dog except K-32 there was a definite rise in the Blood Urea Nitrogen which was followed by a gradual decline. This could be interpreted as being due to the increased amount of regeneration occurring as a result of the carbon tetrachloride induced necrosis.

The average values for the BUN in the 10 dogs over the period of experiment was below the average normal value. Statistically it bordered on significant. The findings for Total Serum Proteins, Serum Albumin and the Albumin-Globulin ratio also showed a significant decrease.

9. Hemoglobin and Hematocrit.

These parameters were used as a guide to hemopoietic activity and health generally. There was a reduction of both, the Hemoglobin decrease being non-significant and the decrease in Hematocrit just significant. However, on examining the table depicting the course of these parameters, the slight but progressive decline is quite evident. Looked upon as a depressive effect on hemopoiesis, the decrease in hemoglobin may be interpreted as further evidence of a depression of

liver function; that is, its hemopoietic function.

10. Mesenteric Pressure

In the 8 dogs in which this parameter was measured, it was significantly raised in every incidence. Although the mesenteric or portal pressure in K-27, the dog which received the largest quantity of carbon tetrachloride, was raised to the greatest height, there did not appear to be any significant correlation between the height of mesenteric pressure, the quantity of carbon tetrachloride administered, or the period of time during which the toxin was given.

This significant increase in portal pressure is interpreted as further corroborative evidence that cirrhosis of the liver was successfully produced in the experimental animals. The mechanism of production of this portal hypertension is not a matter of discussion for this thesis but there seems to be little doubt that it is due largely to disorganization of the hepatic architecture resulting in compression and changes of the vascular arrangement of the liver.

Approximately 75% of the blood flowing through the liver courses through the portal vein. The remaining 25% comes from the hepatic artery at a considerably higher pressure. The portal blood, while poorly oxygenated having already traversed one

capillary bed, nevertheless is rich in nutrient material absorbed from the small intestine. It is not surprising therefore, that the portal venous system should bear the brunt of the obstruction to hepatic blood flow. The pressure in the portal system may be further raised in cirrhosis by arterioportal venous anastomosis which transmit arterial pressure directly to the portal venous circulation. The morphological features characteristic of cirrhosis, in particular nodular regeneration and arteriovenous anastomosis appear to influence hepatic circulation and portal hypertension significantly.

For a long time, fibrosis of the liver in cirrhosis was considered responsible for producing an increase in tissue tension by contracting and constricting the regenerative nodule. (30)

This theory, the result mainly of the classic work of McIndoe has recently been challenged by evidence of the important role of the regenerative nodule in increasing tissue tension, compressing and distorting the hepatic blood supply, augmenting arterioportal anastomosis and producing portal hypertension in the cirrhotic liver. (82,28,83,84)

TEST	AVERAGE RESULTS OF 26 NORMAL DOGS	AVERAGE RESULTS OF 10 EXPERIMENTAL DOGS	STATISTICAL SIGNIFICANCE
SERUM GLUTAMIC OXALACETIC TRANSAMINASE:	46.50 ($\pm$ 3.6)	149.62 units	HIGHLY SIGNIFICANT
SERUM ALKALINE PHOSPHATASE:	6.05 ($\pm$ 0.8)	25.18 K.A. units	HIGHLY SIGNIFICANT
SERUM CHOLESTEROL:	148.54 ($\pm$ 12.6)	135.88 mg. %	NON SIGNIFICANT
SERUM BILIRUBIN:	0.11 ($<$ 0.01)	0.98 mg. %	SIGNIFICANT
BROMSULPHALEIN TEST:	3.28 ($\pm$ 0.35)	27.22 %	HIGHLY SIGNIFICANT
SERUM PROTEINS:			
TOTAL	7.82 ($<$ 0.01)	7.06 g. %	SIGNIFICANT
ALBUMEN	5.39 ($\pm$ 0.16)	3.88 g. %	SIGNIFICANT
GLOBULIN	2.42 ($\pm$ 0.16)	3.20 g. %	NO SIGNIFICANT INCREASE
A:G RATIO	2.49 ($\pm$ 0.102)	1.17 g. %	SIGNIFICANT
CEPHALIN FLOCCULATION:	++ ($<$ +)	+++	SIGNIFICANT
BLOOD UREA NITROGEN:	11.34 ($\pm$ 0.55)	7.58 mg. %	BORDERS ON SIGNIFICANT
HEMOGLOBIN:	17.14 ($\pm$ 1.33)	15.42 g. %	NON SIGNIFICANT
HEMOCRIT:	62.34 ($\pm$ 1.14)	55.21 %	JUST SIGNIFICANT
MESENTERIC PRESSURE:	124.39 ($\pm$ 16.2)	261.88 mm. H ₂ O	SIGNIFICANT

Figures in brackets represent Standard Deviation.

TABLE 2.

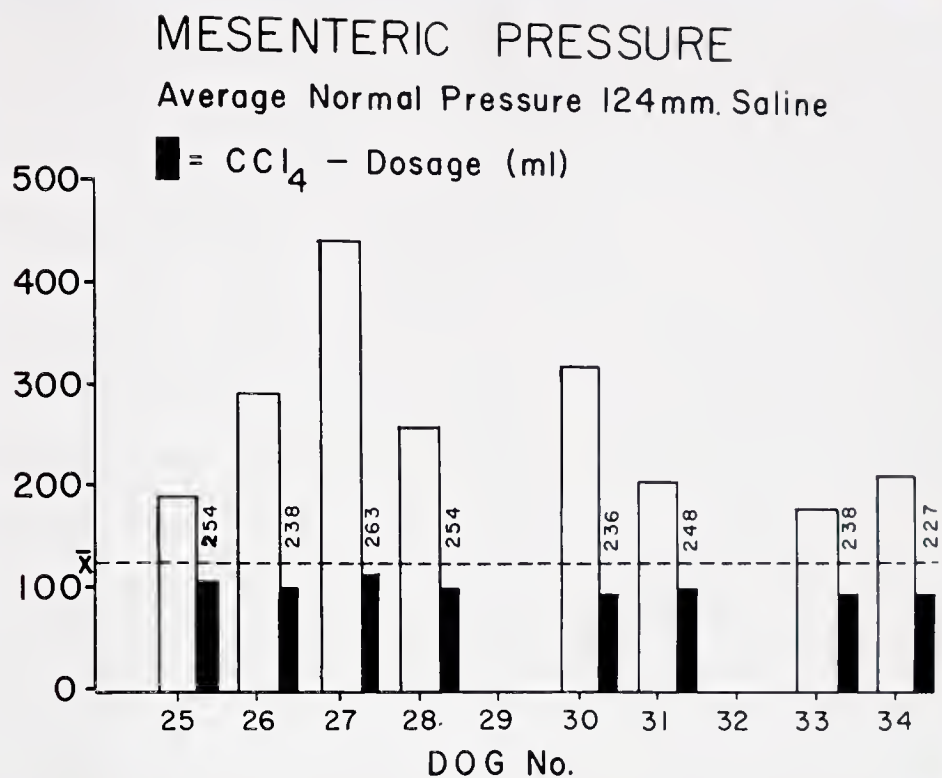


Fig. 179

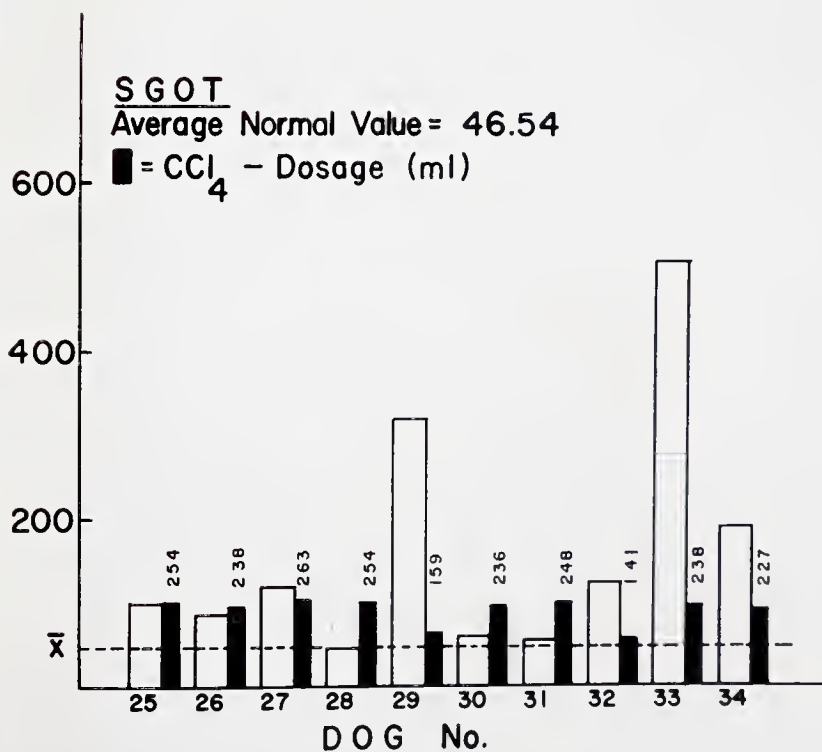


Fig. 180

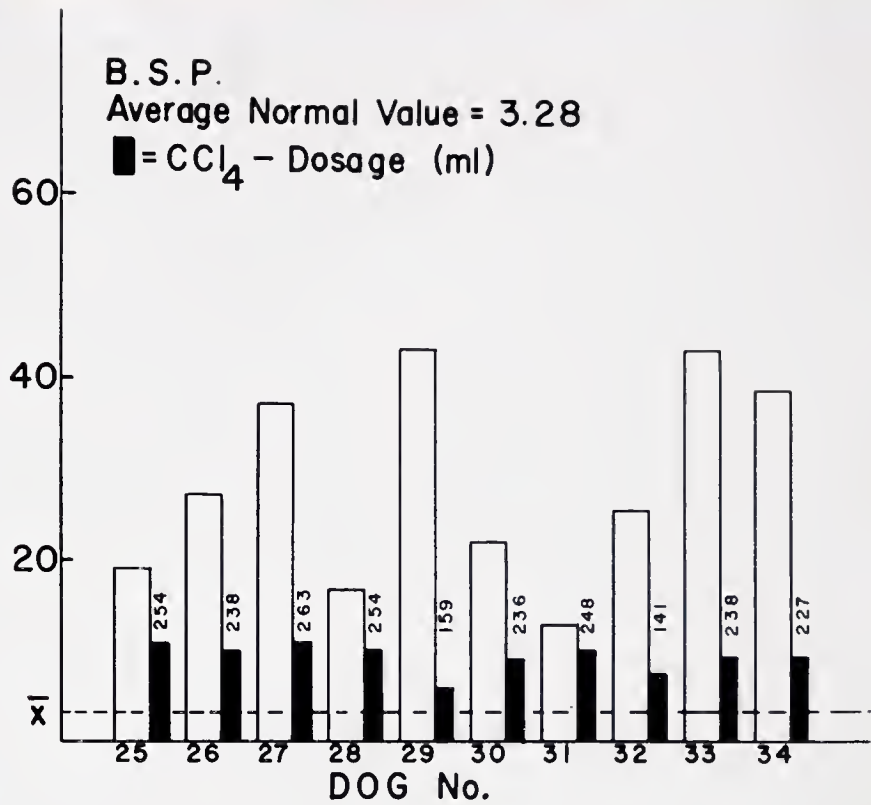


Fig. 181

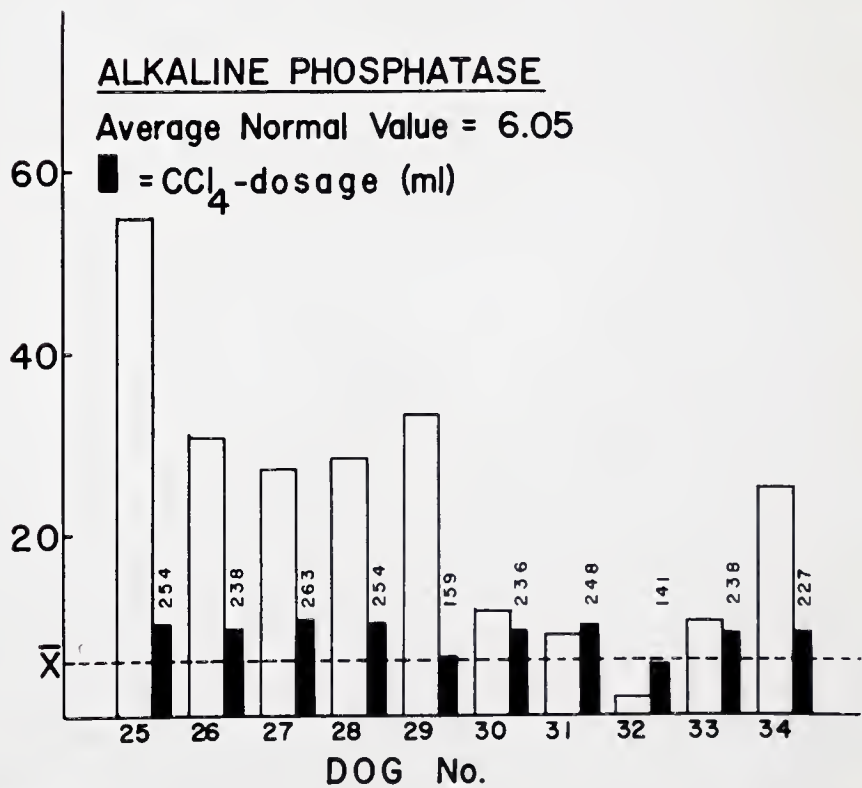


Fig. 182

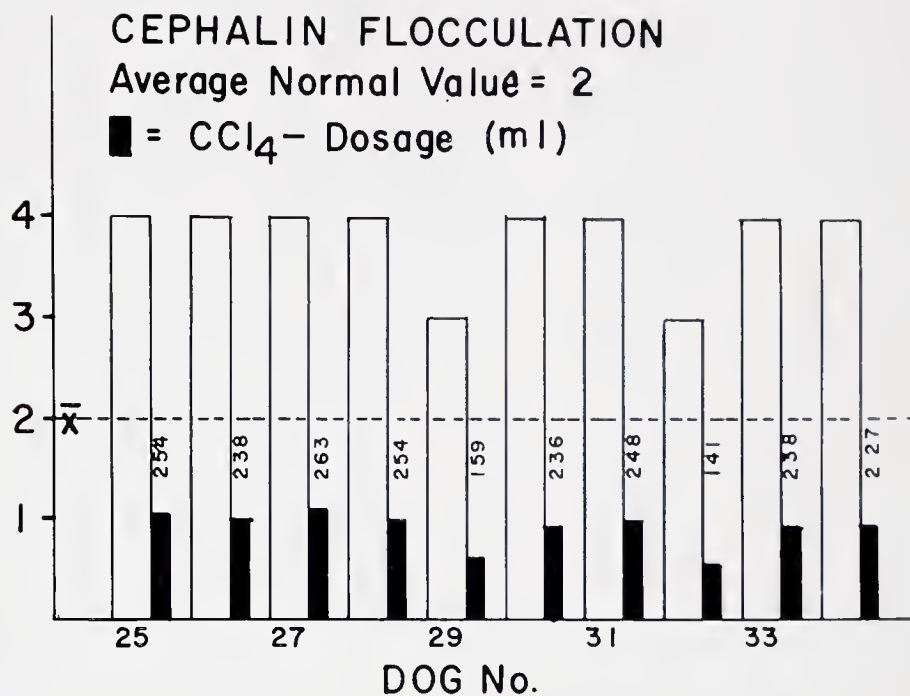


Fig. 183

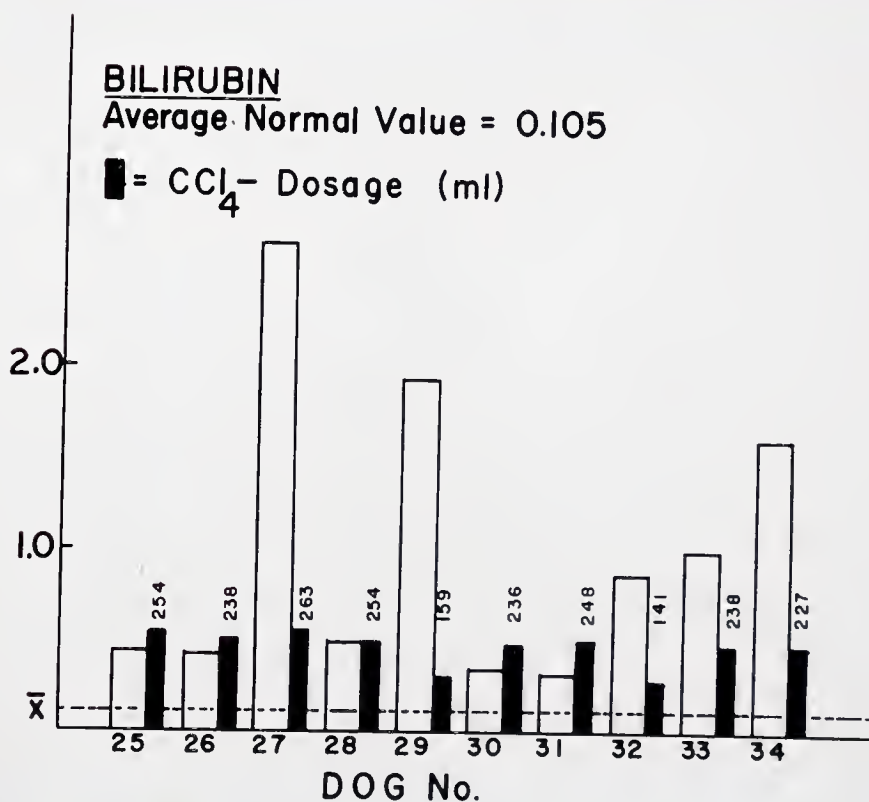


Fig. 184

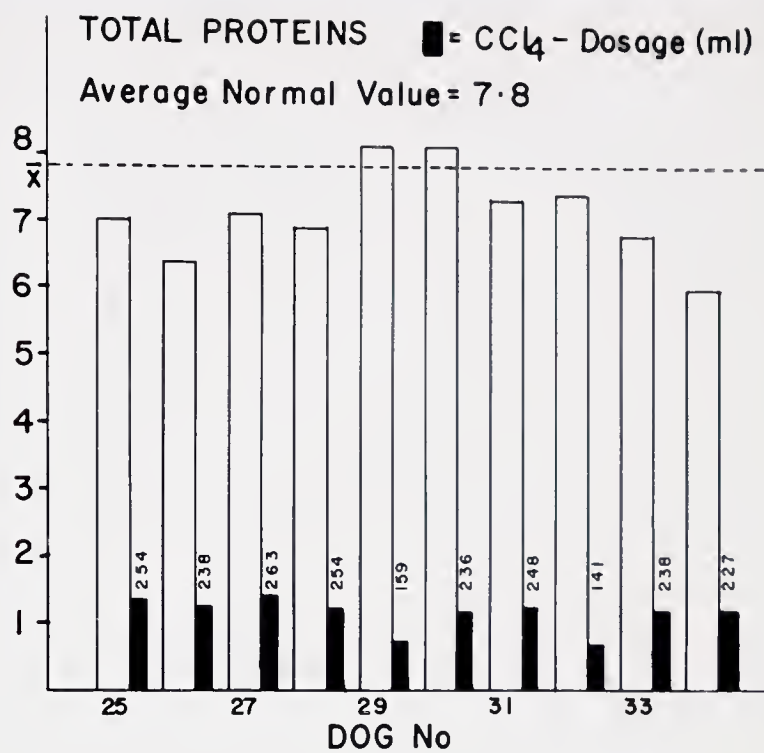


Fig. 185

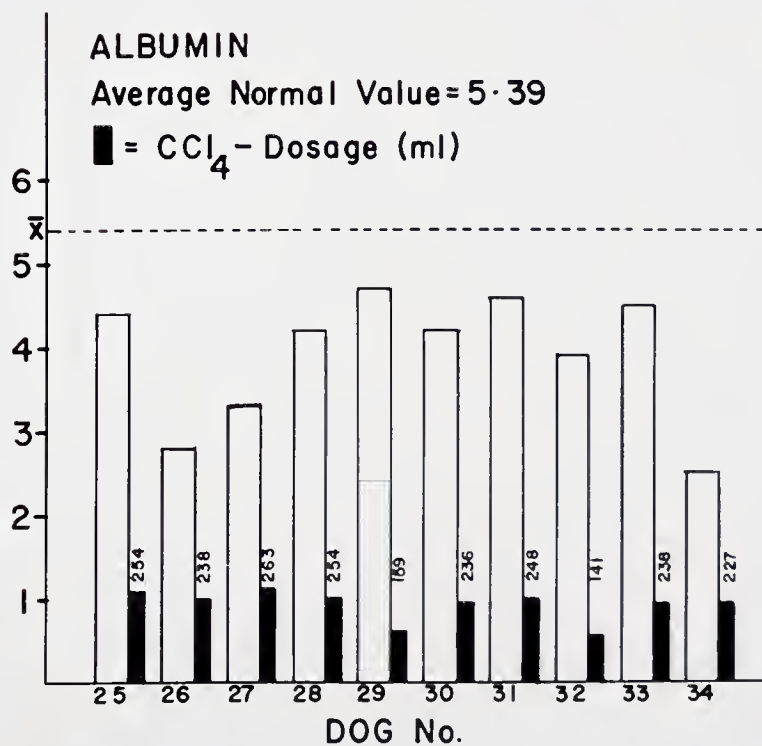


Fig. 186

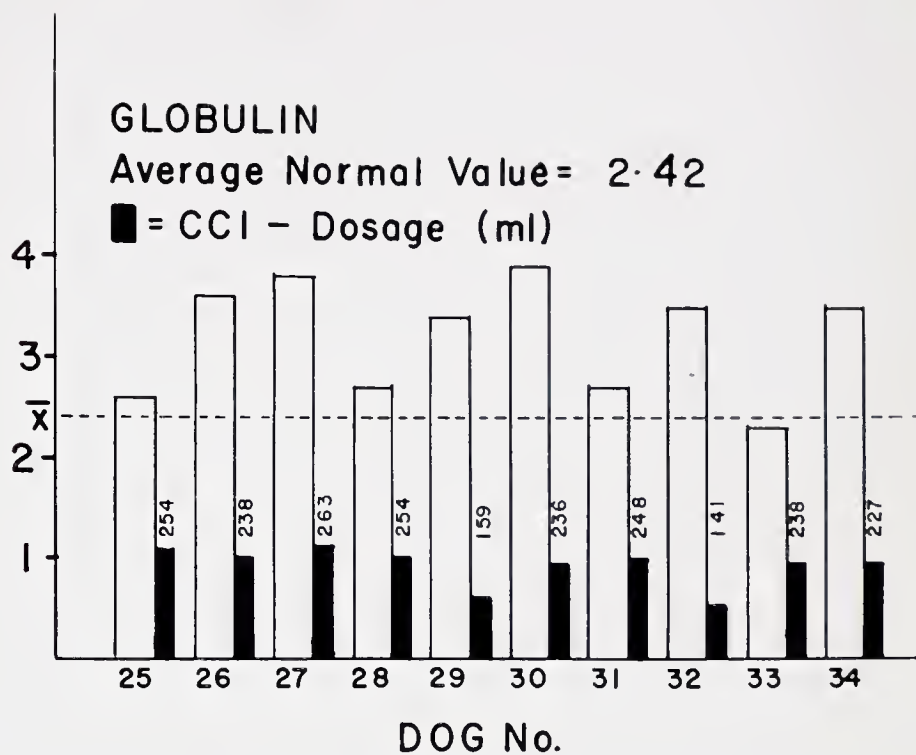


Fig. 187

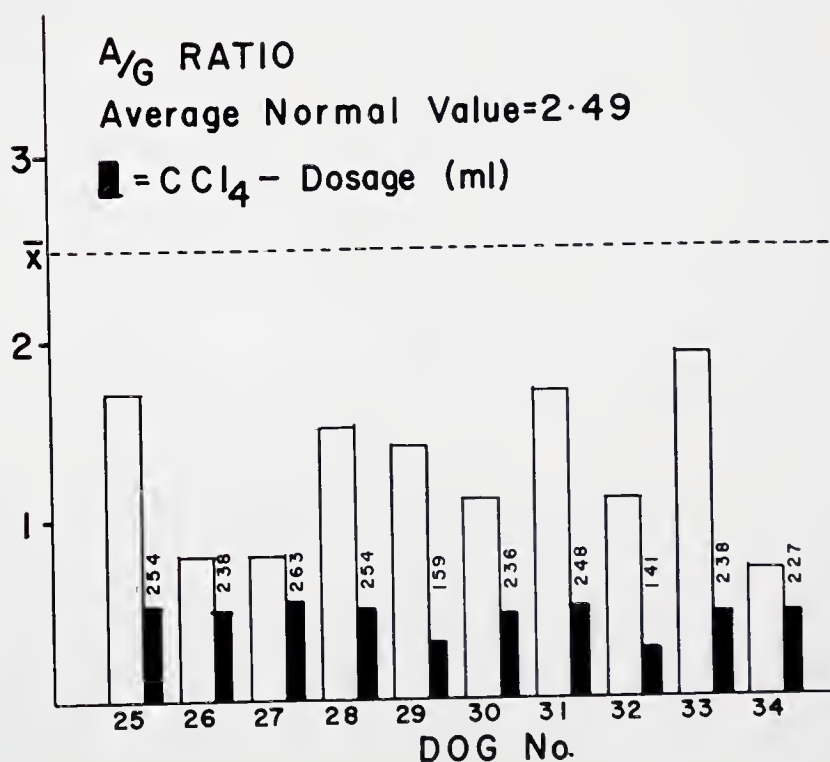


Fig. 188

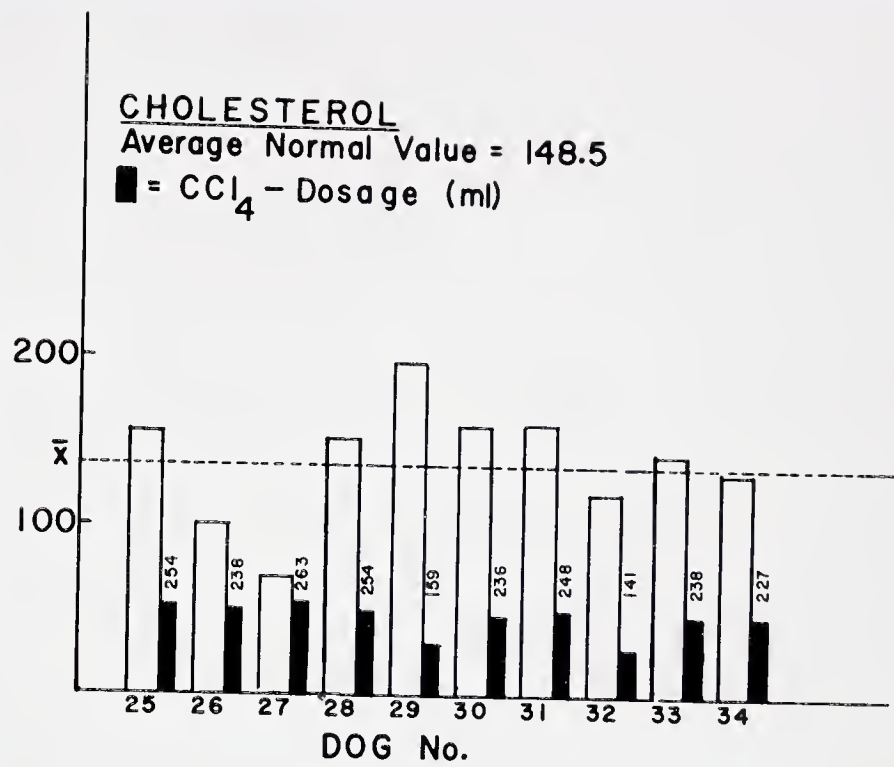


Fig. 189

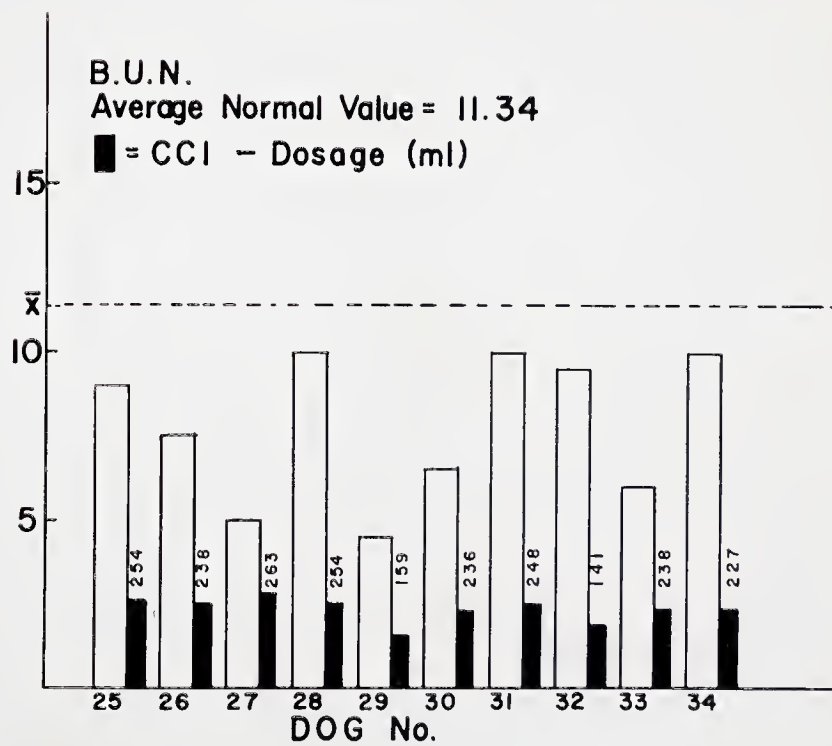


Fig. 190

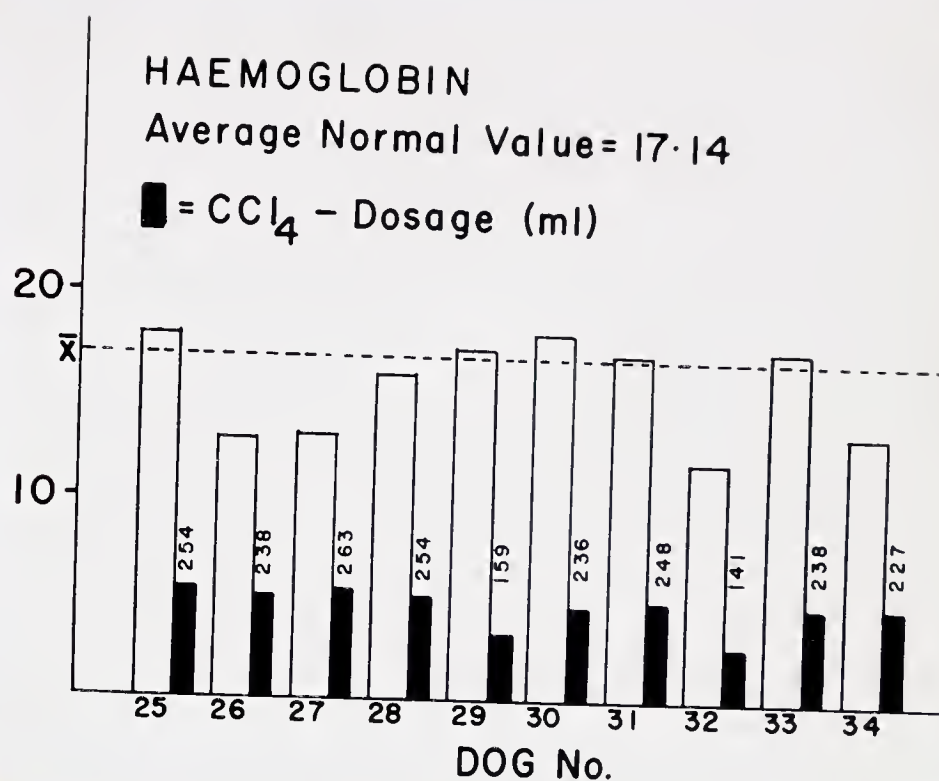


Fig. 191

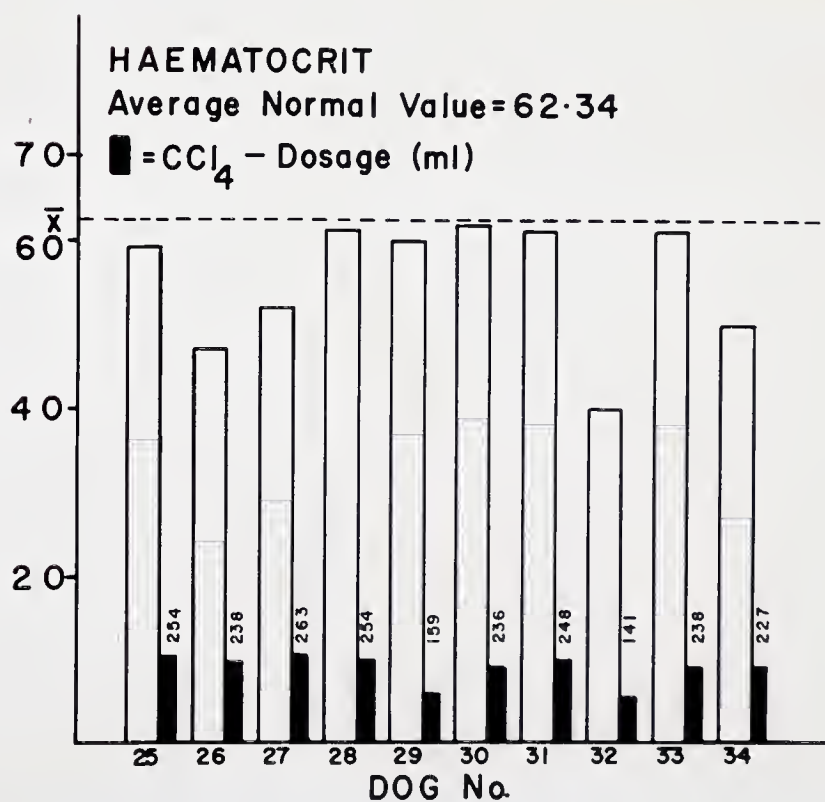


Fig. 192

CHAPTER IV

CONCLUSION

It is possible to produce Portal Cirrhosis and Portal Hypertension rapidly in dogs using oral Carbon Tetrachloride, a chemical which is usually associated, both experimentally and clinically, with the postnecrotic form of the disease. Furthermore it is possible to produce both portal and postnecrotic types of the disease in the same animal using this chemical in the manner described in this experiment.

The ability to produce a different type of pathology than is expected, suggests that factors other than the etiologic agent are at work in the genesis of this disease. The presence of both forms of cirrhosis in the same specimens would appear to confirm this hypothesis.

The necrotic response of the liver to the Carbon Tetrachloride may be guided and monitored to some extent by Serum Glutamic Oxalacetic Transaminase estimates. It must be remembered however that although the response by the animal's liver to the chemical may be followed, it cannot be accurately predicted. It would appear that different animals have differing sensitivity to Carbon Tetrachloride, and that this

sensitivity may fluctuate within the same animal at different times. This may possibly represent another facet of the earlier postulation that the pathogenesis of this disease may vary with factors other than the prime etiological agent.

Serial Liver Function Tests demonstrated a gradually increasing hepatic dysfunction, but one which could not be accurately correlated with the degree of cirrhosis or general condition of the animals. This is postulated to be due to the enormous reserve capacity of the liver to function under adverse conditions, as well as the remarkable regenerative powers demonstrated by this organ.

Ascites occurred in three of the ten dogs used in the experiment. Considering the group as a whole, the occurrence of ascites did not appear to be related to the quantity of Carbon Tetrachloride administered, the length of its period of administration, the level of portal pressure or the state of liver function as interpreted by the biochemical tests performed. However, on examining the data of the three ascitic dogs only, there appears to be a definite correlation between degree of ascites, level of portal pressure, quantity of Carbon Tetrachloride administered and period of administration, in which the more severe degree of ascites is associated

with a higher portal pressure, greater quantity of Carbon Tetrachloride administered, longer period of its administration and more severe degree of liver dysfunction. The Serum Protein estimations in particular suggested a direct relationship between degrees of ascites and liver function

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